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EXPERIMENTS ON PATTERN-VISION OF THE HONEY BEE.

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Introduction.

In my paper on the "Color Vision of the Honey Bee," published in the Biological Bulletin for October, 1910, some experiments were described which, I think, demonstrate conclusively that bees can discriminate between colors. In November of that same year, J. H. Lovell[1] published the results of some researches conducted in a manner quite different from my method; but which led him to form the same conclusion.

On page 277 of my paper is the following paragraph: "How minute are the details that bees observe I am not prepared to say; but that they do observe details is indicated by the following observations: (1) In the boxes used in the experiments of Series IV., the trays of the boxes used were entered, from the portico, by means of an eccentric opening. In most of the boxes this doorway was rectangular, in others it was circular. When a bee first approached one of these boxes, she had to search for the entrance. After a few trips she would land on the portico directly in front of the entrance, and, in some cases, she would fly into the tray without even pausing on the portico. (2) For about an hour bees had been collecting honey from some of the red artifacts. It seemed that nearly all of the bees in that part of the field were collecting from those artifacts. Along side of one of the red boxes I placed a red box on the sides and top of which I had pasted bits of white paper. This gave a box with red front and spotted sides. Into this box I placed some honey. The bees that ap-

[1] Lovell, J. H., "The Color Sense of the Honey Bee; Can Bees Distinguish Colors," *Amer. Nat.*, Vol. XLIV., Nov., 1910, pp. 673–692.

proached this box from the front always entered immediately; the majority of those that approached the sides paused a moment, then went to the nearest red box." This experiment indicated that bees could discriminate between patterns. The purpose of the researches to be described in this article was the establishment of a definite experimental answer to that question.

The method decided upon was to permit a few bees to learn that honey could be collected easier from artifacts of a certain color pattern than it could be from ordinary flowers; and, after that had been thoroughly learned, to see if those bees could select artifacts of that color-pattern from those of a different color-pattern; 1st, when the artifacts to be selected contained honey and the others did not; 2d, when not only the artifacts to be selected but some of the others also contained honey; 3d, when none of the artifacts contained honey.

The artifacts used were constructed out of cardboard and were of three kinds: circular discs about forty millimeters in diameter,

FIG. 1. A few of the artifacts. From left to right: front view of a transversely striped box, side view of a transversely striped box, front view of a longitudinally striped box, side view of a longitudinally striped box.

cornucopias made in the manner described on page 260 of the article published last year, and special experiment boxes made in the manner described on pages 271 and 272 of that article. The artifacts used this year were smaller than those used last. The cornucopias, excluding the lip, were fifty millimeters long and thirty millimeters in diameter at the largest part. The boxes, excluding the lip, were sixty millimeters long, forty millimeters wide and twenty millimeters high. The pattern that distinguished any artifact was placed upon both the inside and the outside. The following colors and color patterns were used: plain red, plain green, alternate red and green longitudinal stripes, alternate red and green transverse stripes, alternate black and

white longitudinal stipes, mottled red and green. With the exception of the red of some of the plain red artifacts, the red and green used on all of the artifacts was of the same hue. The stripes were about five millimeters wide; the spots on the mottled artifacts varied from two to five millimeters in length; they were irregular in shape and arranged in a haphazard manner.

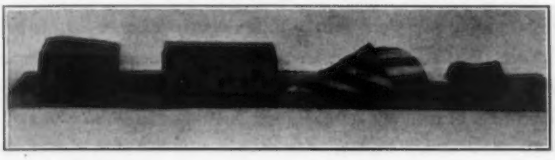

FIG. 2. A few of the artifacts. From left to right; front view of a mottled box, side view of a mottled box, side view of a longitudinally striped cornucopia, end view of a longitudinally striped cornucopia.

In order to have the bees working under normal field conditions, an abandoned meadow was selected as the site of the experiment plot. Most of the field was rank with *Melilotus alba* Lam.; but here and there were patches of almost barren ground. The majority of the experiments were performed in one of these partly barren spots, in which a few scattered mint plants and a few plants of the white sweet clover were growing.

DESCRIPTION OF THE EXPERIMENTS.

Series I. [July 14–17.]

The purpose of this preliminary series of experiments was to permit the bees to learn that honey could be obtained much more easily from artifacts marked with alternate red and green longitudinal lines than it could be from the flowers of the melilot or the mint from which they were foraging.

EXPERIMENT 1. *Early in the afternoon of July 14, two discs, marked with parallel red and green stripes and well supplied with honey, were attached to the branches of a melilotus bush from which bees were collecting honey.*

Although the discs remained on that plant all of the afternoon, no response was made to them by any of the bees.

EXPERIMENT 2. *Three discs, marked with parallel red and green stripes, were attached to melilotus twigs and a large drop of*

honey placed on each. These were placed in position at eight o'clock on the morning of the fifteenth of July.

These discs were watched until two o'clock in the afternoon; but not a bee made any response to them.

EXPERIMENT 3. *At about eight A.M., July 17, 1911, two discs, marked with red stripes on a green back-ground were attached to the tops of upright sticks placed in the midst of a mint weed from which several bees were collecting honey. By means of a string the branches of the plant were drawn together in such a manner as to have several blossoms near each of the discs. Honey was placed on the discs and on some of the blossoms near by. Accidentally some honey fell upon some of the leaves.*

After waiting, restlessly, in the hot sun, for nearly two hours, I was rewarded for my patience by seeing a bee alight on one of the discs, and, while hanging suspended from its edge, sip the honey. When it had finished and before it started for the hive, the bee made a short flight of orientation. A few minutes later a bee alighted on the top of disc number two and began to sip the honey. Before departing for the hive, it made a flight of orientation. From this time to the close of the experiment, thirty-five visits were made to the discs, twenty to disc one and fifteen to disc two. Often bees were on both discs at the same time. On one occasion, three bees were on disc two and two on disc one. On another occasion, two bees were on each disc. Soon after the bees began to collect from the discs I noticed that several bees were collecting honey that had dropped on the leaves. To check them, wherever I saw honey on a leaf I covered it with dust. By this means I hoped to cause those bees to notice the honey on my artifacts. The habit of collecting from leaves had become fixed and those bees spent the remainder of the afternoon searching the leaves for honey. Occasionally they would find a new supply and collect therefrom until I covered it with dust. By the close of the afternoon, about six bees were collecting from the artifacts and four hunting for honey among the leaves.

EXPERIMENT 4. *About two hours before departing for home, disc two was placed in a new position and a cornucopia marked with alternate red and green longitudinal lines was placed where*

it had been. Disc one was left in its original position. All of these artifacts were well supplied with honey.

Up to the close of the experiment, eleven visits were made to the artifacts; four to disc number one, three to disc number two, and four to the cornucopia.

EXPERIMENT 5. *Half an hour before departing for home, disc number one was placed in a new position and a second cornucopia, resembling number one in its color pattern, was placed in the place rendered vacant by the removal of disc number one. Cornucopia number one and disc number two were left in the positions occupied in experiment 4. All of the artifacts were well supplied with honey.*

The bees made twelve visits to these artifacts; four to disc number one, two to disc number two, three to cornucopia number one, and three to cornucopia number two.

Experiment number three was begun at about eight A.M., experiment number five closed at five P.M. Throughout the whole of that interval the artifacts were observed continuously.

On leaving for home all of the artifacts were well supplied with honey and left in the field.

The bees collecting from those artifacts were only a small per cent. of those visiting that part of the field; but they behaved so unlike the others that I could recognize one as soon as it arrived within a yard of the artifacts. The bees that had not begun to collect honey from my artifacts or honey-smeared leaves, on reaching the experimental plot, would simply wander from flower to flower, pausing a moment at some and simply glancing at others. On the other hand, bees that were collecting honey from my artifacts or honey-smeared leaves, on reaching the plot, would describe a number of complex, irregular, number eight curves, no two of which seemed to be in the same plane and which became gradually shorter and shorter until the artifact or honey-smeared leaf was found by the bee. To one looking on, it was almost impossible to resist the belief that those bees were seeking some definite thing or things.

Series II. [July 18, 1911.]

On reaching the field, at about eight thirty A.M., there was no honey in any of the artifacts; but the bees were moving in and

out of the cornucopias as though they were hunting for something.

EXPERIMENT 6. *One of the cornucopias used in the last experiment was replaced by an experiment box marked with alternate red and green longitudinal stripes. The other cornucopia was left in place and called striped cornucopia number one. Three groups of three short stakes each were erected near the plant containing the two stakes mentioned above. The groups were about a foot apart and the stakes in each group separated by an interval of about two inches. The groups were numbered from two to four, two being the nearest to the two stakes and four being the most remote. The stakes were arranged in a section of a curve. On the top of one of the stakes in each group a cornucopia marked with alternate red and green stripes was placed, while a similar plain red and plain green cornucopia crowned each of the other stakes. In no two groups was the arrangement of the cornucopias the same. In the striped box and in the striped cornucopias, honey was placed. The other artifacts were empty. The position of the artifacts remained the same throughout the experiment.*

This experiment began at about nine A.M. and extended until noon. Except during the time consumed in taking a photograph, the artifacts were watched continuously. Not a single visit was made to either the red or the green cornucopias; but forty-seven entrances were made into the cornucopias that were marked with alternate red and green longitudinal stripes. These visits were distributed as follows: striped box number one, nine; striped cornucopia number one, twenty-one; striped cornucopia number two, eight; striped cornucopia number three, seven; striped cornucopia number four, two. Thus all of the striped artifacts were visited. Sometimes a bee would enter one artifact and then leave and enter another and sometimes yet another. It usually sipped honey in the last visited artifact only. In some cases the cause of leaving the first artifact was the presence of some bee; in most cases, however, I could not discover the cause for departing.

EXPERIMENT 7. *A fourth group of three stakes was added to the above group and called group five; it was arranged about half way, in a straight line, between group two and group four. All of the*

cornucopias were replaced by experiment boxes of the same color patterns. At irregular intervals, the arrangement of the boxes in each group was altered. All of the boxes marked with alternate green and red longitudinal stripes contained honey, the other artifacts were empty.

This experiment extended from noon to five P.M. Except while taking a photograph, the artifacts were watched continuously. During that time not a single entrance was made into either the green or the red boxes; but eighty-nine entrances were made into the striped boxes; and each such box was visited several times. The visits were distributed as follows: striped box number one, eight; striped box number two, fifteen; striped box number three, twelve; striped box number four, seven; striped box number five, twenty-one.

One of the cornucopias used in this experiment leaked and formed, on the slightly inclined stake, a line of honey. Several bees discovered this honey and began to carry it to the hive. The line of honey was on the side of the stake that was turned away from me and I did not know that the bees had formed the habit of collecting honey from the side of the stake until experiment seven was half over. I saw bees flying to that side of the stake; but I supposed they were some of the bees who had formerly formed the habit of collecting honey from smeared leaves, and, since I had seen to it that there was no honey on any of the leaves, I did not give them much attention until I noticed that bees were frequently flying from that place to the hive. As soon as I realized that they had formed that habit, I covered the honey with road dust, hoping to induce the bees to attend to my artifacts. Instead of entering the artifacts, they continued to examine the stake; occasionally one would fly to another stake and examine it. This was not because they had not noticed the artifacts, but because the artifacts had acquired no meaning to them. They would frequently approach one of the boxes containing honey and hover before it momentarily and then return to searching the stakes. To them the stakes had come to mean "honey-bearers" and they seemed to use the artifacts and neighboring plants as reference points.

Most of the bees continued to describe those complicated num-

ber eight curves, whenever they arrived at the experiment plot; but the number of curves described were perceptibly fewer and the lengths much shorter, and some of the bees seemed to fly practically direct to the artifacts.

On departing for home, all of the artifacts, except three striped boxes, were removed. Those boxes were well supplied with honey.

July nineteenth, I arrived at the field at 9:30 A.M. and found the boxes empty and the bees flying into and out of them as though hunting for something. It began to rain soon after my arrival, which made it impossible to continue my experiments that day. On leaving, all of the artifacts were removed from the field.

Series III. [July 20, 1911.]

The morning was quite cloudy and when I arrived in the field, at about half past seven, the bees were not foraging.

EXPERIMENT 8. *Three groups of three experiment boxes each were arranged in the manner described above. In each group there was one box marked with alternate red and green longitudinal stripes, one green box and one red box. The order of the boxes was different in each group. When the experiment was about half over, the arrangement of the boxes in each group was changed. All of the boxes marked with alternate red and green longitudinal stripes contained honey; the others were empty. The experiment was an hour long.*

Twenty-five entrances were made into the interior of the striped boxes; but not a single entrance into either the red or the green boxes. The visits were distributed as follows: striped box number one, ten; striped box number two, eight; striped box number three, seven. Only once did a bee, on arriving at the plot, make the irregular number eight curves mentioned above. On arriving at the plot, the bee would either fly directly to one of the striped boxes, or else fly along, examining box after box, until it reached a striped box. On leaving, the bees did not always make flights of orientation. Often a bee would arrive at the hind end of one of the experiment boxes that contained honey and attempt to find an entrance; then it would sidle around to the front end and enter.

EXPERIMENT 9. *Three groups of boxes were arranged as in*

experiment eight; but honey was placed, not only in the striped box, but also in red box number one and green box number two. When the experiment was about half over, red box number one was placed where striped box number one had been and striped box number one placed on the stake made vacant by the removal of red box number one; and green box number two was placed where striped box number two had been and that striped box placed on the stake made vacant by the removal of green box number two. The time occupied was about one hour.

Twenty-two entrances were made into the striped boxes, half before and half after the change was made in the arrangement of the boxes; but not a single visit was made into the interior of either the red or the green boxes, although one box of each of those colors contained honey. The visits were distributed as follows: striped box number one, thirteen; striped box number two, four; striped box number three, five. One bee hovered before the hind end of red box number one for several seconds and then flew to striped box number one. Another bee alighted on the front end of red box number one and then departed. Bees often paused momentarily before all of the boxes; but they never entered any that were not marked with alternate red and green longitudinal stripes.

EXPERIMENT 10. *Three groups of boxes were arranged as in experiment eight; but no honey was placed in any of the boxes and none had ever contained honey. The duration of the experiment was about fifteen minutes.*

Fourteen entrances were made into the striped boxes; but not a single visit was made to the interior of either the green or the red boxes. The visits were distributed as follows: striped box number one, six; striped box number two, three; striped box number three, four.

EXPERIMENT 11. *Two groups of artifacts were removed from the field; thus leaving only one group of boxes, all of which were empty and none of which had ever contained honey. The time allowed was about ten minutes.*

Ten entrances were made into the striped box, but not a single visit was made to the interior of either of the other boxes. When I terminated the experiment, about six bees were hovering about the striped box, examining it carefully.

Series IV. [July 21, 1911.]

EXPERIMENT 12. *Two sets of five boxes were arranged on stakes. In each group there was one of each of the following artifacts: box marked with alternate red and green longitudinal stripes, box marked with alternate black and white longitudinal stripes, box mottled with green and red, a plain red box, and a plain green box. The arrangement of the boxes in each group was different, and was changed twice during the experiment. The box marked with alternate red and green longitudinal stripes was supplied with honey; the others were empty. The time consumed was about one and a half hours.*

Sixty-three entrances were made into the boxes marked with alternate red and green longitudinal stripes and one into the boxed mottled with green and red; no visits were made into the interiors of any of the other boxes. Bees frequently hovered before other boxes and twice bees alighted on the portico of the box marked with alternate black and white longitudinal stripes, but did not enter. The visits to the artifacts marked with alternate red and green longitudinal stripes were distributed forty-two to box one and twenty-one to box two.

EXPERIMENT 13. *One set of six boxes was arranged on stakes. To the kinds of boxes used in experiment 12, I added a box marked with alternate red and green longitudinal stripes. The box marked with longitudinal red and green stripes was supplied with honey; the others were empty. Starting at the left, the arrangement of the boxes was as follows: plain green, marked with alternate red and green longitudinal stripes, mottled with green and red, marked with alternate black and white longitudinal stripes, plain red, marked with alternate red and green transverse stripes. The time consumed was about half an hour.*

Nineteen entrances were made into the box marked with alternate red and green longitudinal stripes; but no visits were made to the interior of any other box.

EXPERIMENT 14. *The same number and kinds of boxes were used as in experiment thirteen; but the mottled red and green box was placed where the box containing honey had been and the box containing the honey was placed on the stake rendered vacant by the removal of the mottled box. The time consumed was about half an hour.*

Twenty entrances were made into the box marked with alternate red and green longitudinal stripes and one into the box that was mottled with red and green. No visits were made into the interior of any other box.

EXPERIMENT 15. *The same number and kinds of boxes were used as in experiment fourteen; but the box marked with alternate red and green transverse stripes was placed where the box containing the honey had been, and the box containing the honey was placed where the box marked with alternate red and green transverse stripes had been. The time consumed was about three-quarters of an hour.*

Thirty-six entrances were made into the box marked with alternate red and green longitudinal stripes, six into the box marked with alternate red and green transverse stripes, and two into the box marked with alternate black and white longitudinal stripes. No visits were made into the interior of any other box. The bees that selected the wrong boxes did not enter them as promptly as did those that entered the right box. In each case the bee hovered before the entrance quite a while before entering. All of these mistakes were made during the first ten minutes of the experiment.

EXPERIMENT 16. *Two boxes marked with alternate red and green longitudinal stripes and two boxes marked with alternate red and green transverse stripes were arranged on stakes, in a line, with all of the entrances facing in the same direction. For the first half of the experiment the different kinds of boxes alternated; during the latter half, the boxes with the longitudinal stripes were in the middle. The boxes with longitudinal stripes contained honey, the others were empty. The time consumed was about half an hour.*

The purpose of this experiment was to accustom the bees to ignoring the transversely striped boxes. Thirty-three entrances were made into the longitudinally striped boxes and none into any of the others.

EXPERIMENT 17. *The same number and kinds of boxes were used as in experiment fifteen and they were arranged in the following order: box marked with alternate black and white longitudinal stripes, box marked with alternate red and green transverse stripes, box marked with alternate red and green longitudinal stripes, plain green box, box mottled with red and green. The box marked with*

longitudinal red and green stripes contained honey; the others were empty. The time consumed was about fifteen minutes.

Eleven entrances were made into the box marked with alternate red and green longitudinal lines; no entrances were made into any of the other boxes.

EXPERIMENT 18. *The same kind and number of boxes were used as in the above experiment, but they were arranged in the following order: box marked with alternate black and white longitudinal stripes, box marked with alternate red and green transverse stripes, plain green box, box marked with alternate red and green longitudinal stripes, box mottled with red and green, plain red box. None of the boxes contained honey or ever had contained honey. The time consumed was about fifteen minutes.*

Thirty entrances were made into the box marked with alternate red and green longitudinal stripes, but no visits were made into the interior of any other box.

EXPERIMENT 19. *An empty box marked with alternate red and green longitudinal stripes and which never had contained honey was placed on the palm of my hand and by its side was placed an empty box marked with one of the other patterns. The entrances of both boxes were made to face the same way. All other artifacts were removed from the field and my hand held near the stakes upon which artifacts had rested. At the end of about two minutes the position of the boxes was altered. This maneuver was repeated with each kind of box used in experiment eighteen.*

At least a hundred entrances were made into the box marked with red and green longitudinal stripes; but not a bee entered any of the other boxes. One bee acted as though it was going to enter the box marked with alternate red and green transverse stripes, but it did not do so. Towards the end of the experiment the bees entered the box marked with alternate red and green longitudinal stripes so rapidly that it was almost impossible to keep an accurate record. To one looking on, it seemed as though the bees were looking for something which they expected to find in those boxes.

When I left for home the bees were searching over and around the barren stakes for something that was not.

Interpretation of the Experiments.

These experiments furnish additional evidence, if such is needed, that Plateau is mistaken when he claims that odor is the sole factor that leads bees to the honey of flowers; for my discs, marked with red and green stripes and well supplied with honey, remained exposed to bees for fully two days before a single bee collected honey from them [Ex. 1–5].

These experiments strengthen the impression, gained from previous work with hymenopterous insects, that such insects can be induced to form new associations that will influence their behavior, if only the experiment is so planned as to lead the insect to accidentally make the association. So far as my experience goes, it is almost impossible to coerce them into forming new associations, and an abundance of time is often required for the formation of chance associations. Lack of response to a stimulus does not mean that that stimulus has not been noticed by the insects; but that, to them, it has not yet acquired a meaning.

In inducing bees, in the field, to respond to new food-objects, the new associations are formed much more quickly when the food is exposed to view than when it is hidden. They may be induced to form such associations, however, even under the latter conditions. These statements are supported by the fact that last year, when I conducted a lengthy series of experiments with discs and cornucopias, artifacts which did not hide the honey from view, I soon had more bees responding to the artifacts than I could keep an accurate record of; whereas this year, when I laid aside my discs and cornucopias as soon as about half a dozen bees had made the association, at no time were more bees at work than could be counted. At the close of the series about twice as many bees were collecting from the artifacts as at the beginning.

When a bee had discovered one of my honey-producing artifacts and collected therefrom, it would make a flight of orientation and then fly home. On returning to the field it would, by means of a series of ever narrowing, complex, figure eight curves, search out the experiment plot, and then, by a closer scrutiny of the surrounding objects, locate the artifact desired. [Last

paragraph of Series I.] After a bee had made several trips to the plot, it would fly directly to it without making the complicated searching movements. This striking behavior furnished a sure means of telling when a new bee had formed the association. I changed the arrangement of the individual artifacts so often that the bees usually were forced to search for the honey-producing box. After the association had been well established, the bees usually departed for home without making a careful flight of orientation. If, however, I had made a marked change in the position of the artifact since the last visit of the bee, then a careful flight of orientation was always made. No one who had watched these pronounced searching movements could doubt that bees are largely guided by memory pictures of the environment.

After a bee had learned, by experience, that artifacts bearing a certain color pattern contained a more copious supply of easily obtained honey than ordinary flowers, it would select artifacts bearing that color pattern from those marked in a different way. This was true: (1) when several of the artifacts to be selected were scattered among a number of plain artifacts of the colors used in making the color-pattern [Ex. 6–11]; (2) when the artifact to be selected was scattered among several other artifacts, some of which were plain and some of which were marked with patterns unlike that of the artifact to be chosen [Ex. 12–15, 17, 18]; (3) when the only difference between the artifacts was that one was marked with transverse and the other with longitudinal stripes [Ex. 16]; (4) when the artifact to be selected contained the honey and the others did not [Ex. 8, 12–17]; (5) when honey was to be found not only in the artifact to be selected, but in some of the other artifacts also [Ex. 9]; (6) when none of the artifacts contained honey [Ex. 10, 11, 18, 19].

All of the facts mentioned in the above paragraph are recorded in the following table.

Although this table shows conclusively that bees can recognize color patterns it does not tell the whole story. It does not show that two of the mistakes were made in selecting a box mottled with red and green for one marked with longitudinal red and green stripes; that two mistakes consisted in selecting a box marked with black and white longitudinal stripes for one marked with

TABULATED RESPONSES OF HONEY BEES TOWARDS PATTERNS.

The bee to select an artifact marked with alternate red and green longitudinal stripes when associated with	No. of Trials.	No. of Successes.	No. of Failures.	Per Cent. of Successes.
Plain red and plain green artifacts, an equal number of the three kinds being used; when the artifact to be selected contained honey and the others did not	161	161	0	100.0
Plain red and plain green artifacts, an equal number of each of the three kinds being used; when the artifacts to be selected and some of each of the other kinds contained honey	22	22	0	100.0
Plain red and plain green artifacts, an equal number of each of the three kinds; none of the artifacts contained honey	24	24	0	100.0
Artifacts marked with alternate red and green transverse stripes; the artifacts to be selected contained honey, the others were empty	33	33	0	100.0
Two of each of the following artifacts: plain green, plain red, mottled red and green, marked with alternate black and white longitudinal stripes; the artifacts to be selected contained honey, the others did not	64	63	1	98.5
One of each of the following artifacts: plain green, plain red, mottled red and green, marked with alternate black and white longitudinal stripes, marked with alternate red and green transverse stripes; the artifact to be selected contained honey, the others were empty	84	75	9	89.3
One of each of the following artifacts: plain green, plain red, mottled red and green, marked with alternate black and white longitudinal stripes, marked with alternate red and green transverse stripes; none of the artifacts contained honey	30	30	0	100.0
Any one of the artifacts mentioned in the rectangle immediately above this one; the bees being given an equal number of chances at each of the five pairs; none of the artifacts contained honey	100	100	0	100.0
Grand total	518	508	10	98.5

red and green longitudinal stripes, and that the other six mistakes consisted in selecting a box marked with red and green transverse stripes for one marked with red and green longitudinal stripes. And it does not show that the bees soon learned to avoid making even these mistakes.

There was much in the behavior of these bees to indicate that, in their ability to distinguish minute details, they are near sighted. On examining the artifacts, either when searching for an artifact or in making its flight of orientation, the bee always hovered within about a centimeter of the object examined. The first box a bee reached, on arriving at the plot, was usually not the desired one, and, after a momentary examination, it would pass on to the next.

Evidently bees can distinguish between color-patterns, and this is of value to them in recognizing plants that yield honey. Hence, since insects can distinguish colors and the fine details of color pattern, there is nothing about the visual powers of bees that militates against the theory that the colors and the color markings of flowers are adaptations to insect visitors.

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July 22, 1911.

PHOTOTACTIC REACTIONS AND THEIR REVERSAL IN THE MAY-FLY NYMPHS HEPTAGENIA INTERPUNCTATA (SAY).

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Reactions to Light.

When nymphs of the may-fly *Heptagenia interpunctata* are placed in a long glass dish of water near a window, they immediately swim away from the light, and upon reaching the end of the dish they repeatedly collide with and claw against the invisible obstruction. These movements may be kept up, with intervals of rest, for a whole day. When quiet, the nymphs do not always face away from the light, nor when they swim do they always go in a straight line away from it. Often if there are large dark objects in the room, they will swim against the side of the dish in the direction of the objects.

As the behavior of the nymphs is often much affected by objects in the neighborhood, most of the experiments on the reactions to light were performed in a dark room in a large, evenly colored box constructed of white cardboard, thus shutting off all objects and undesirable light. The light usually employed was derived from an ordinary sixteen candle power incandescent lamp attached to a wire and movable about at will. Almost without exception, the nymphs swam away from the light. Occasionally, some specimens after repeatedly clawing against the transparent barrier and not securing a hold would fall down on their backs with their legs sticking up, remaining in that position sometimes for hours. If a stone was placed in the end of the basin nearest to the light, the nymph would attach itself and remain there for days. When the light was brought near the stone and moved about, the insect would seek the shaded area, but light even much more intense would seldom force it to desert the stone entirely.

When a piece of white cardboard is placed on the water in a small glass dish, the nymphs soon attach themselves to the lower

side of it. By illuminating that side of the paper they soon crawl upon the top which is shaded. Then, when the strong light is again thrown from above, they move below, and in that manner can be made to move back and forth repeatedly, though not with such precision after the experiment is kept up for some time. The nymphs respond to light most readily when occupying the upper side of the paper, and the best results are obtained when white paper is used. Quite similar results are obtained by using gray or black paper, but in such cases the nymphs take a much longer time to move from one surface to another.

I have performed quite a number of series of experiments with different groups of nymphs using two lights, differing in intensity, one at each end of a glass basin twenty-four inches long. I noticed that by placing the nymphs in the water half way between the two lights they would often continue going in the direction in which they first started, regardless of the intensity of the light, though many of them manifested much promptitude in going towards the end of the dish from whence came the light of less intensity, even when there was a difference of only a few candle powers. The excitability apparent in many specimens at the instant of being placed in the water, which is largely responsible for their reckless behavior was almost totally reduced by placing the nymphs in a narrow tunnel made of glass strips, which was placed in the middle of the large basin at right angles to the rays of the two lights. The tunnel was sufficiently wide to enable the specimens to go forward, but not wide enough to enable them to turn around in it. Then the nymphs were put in at one end and allowed to pass through two or three inches of the tunnel, thus getting the full force of the two lights at the same time, and also a chance to resume a more normal attitude. By the time the end of the tunnel was reached, which was in line with the two lights, it was only a matter of choice which direction was to be taken. Sometimes the nymphs would manifest no choice, but remain in the middle of the dish for several minutes, especially when the two lights differed little in intensity. But, generally the insects evinced considerable precision in choosing the end of the basin from which came the light of less intensity. The results obtained in some of the experiments are given in the following tables.

TABLE I.

Series.	Turns Toward 80 c.p.	Indifferent.	Turns Toward 16 c.p.	Total.
1	0	0	10	10
2	0	0	10	10
3	0	0	10	10
4	0	0	10	10
5	0	0	10	10
6	0	0	10	10
Totals	0	0	60	60

TABLE II.

Series.	Turns Toward 32 c.p.	Indifferent.	Turns Toward 16 c.p.	Total.
1	0	2	23	25
2	0	0	25	25
3	1	3	25	25
4	2	0	23	25
5	0	0	25	25
6	1	1	23	25
Totals	4	6	140	150

TABLE III.

Series.	Turns Toward 10 c.p.	Indifferent.	Turns Toward 16 c.p.	Total.
1	1	2	7	10
2	1	3	6	10
3	0	3	7	10
4	0	4	6	10
5	2	3	5	10
6	1	3	6	10
Totals	5	18	37	60

In further experiments an apparatus essentially the same as that used by Strasberger in his work on *Protista* was employed. It consisted of a prism of a solution of India ink placed over a long glass trough the sides and bottom of which were covered over with white paper. Ten specimens were placed in the end of the trough under the thin portion of the prism, and when fairly quiet the light was reflected perpendicularly through the prism giving a perfect gradation in the intensity of light from one end of the trough to the other. Three of the specimens immediately swam toward the dark end of the trough; four others followed within half a minute; and the remaining three collected into a group remaining that way for a few minutes when they

were separated, and within another minute also left for the dark area. Sometimes a specimen would return to the less obscure end of the dish and not finding anything favorable to its thigmotactic inclination, it went back whence it came from. This experiment was repeated several times, always with similar results.

The light was next permitted to enter the trough obliquely, the thicker end of the prism being next to the light. Again five of the individuals made for the darker end of the trough almost immediately, this time going against the rays of the light. About half a minute later two other specimens followed and within three minutes all had deserted the light area. This experiment was repeated many times with ten different sets, and always in less than one minute a large majority of the specimens were in the dark portion of the trough. There were almost always a few slow specimens which required a longer time, from one to ten minutes and even much longer, to be moved. If left in the trough, almost invariably at the end of a few hours the nymphs, with some exceptions, would gather into a group in the obscure region and remain there indefinitely. If the prism and light were reversed, leaving the entangled colony undisturbed in the more illuminated end, the colony would slowly break up and within several hours again form in the more obscure territory.

Another apparatus was so arranged that the length of the prism could be increased or lessened by tipping the trough holding the solution, at various angles, and thus augmenting or diminishing the contrast between the two ends of the trough. It was observed that the proclivity of most nymphs to choose one end of the dish in preference to the other varied directly in proportion to the contrast in the illumination of the two extreme regions of the trough. Here again, we find that some individuals react far more readily than others.

It was also observed that some nymphs under ordinary light manifest no signs of agitation when in a uniformly lighted area, yet when an area in their neighborhood becomes slightly shaded they soon aggregate there.

Control of Phototactic Reactions by Chemicals.

It was found in the experimental work on the reactions to light that *H. interpunctata* nymphs are practically all negatively

phototactic, but that the different individuals vary in the intensity of their negative response. Also, out of about five or six hundred specimens several were quite indifferent, and a few were even weakly positive. This obvious variability in response among the different individuals, in an identical external environment, is apparently due to the variability in the internal conditions of the various specimens.

Loeb[1] says that in all probability light produces chemical changes in the eye or skin of the animals, and that these changes are responsible for the heliotropic reactions. In a brief preliminary communication[2] on the control of heliotropic reactions in fresh water crustaceans by chemicals, he says that specimens of *Gammarus pulex*, which are naturally negatively heliotropic can be made positive by specific chemical substances. For example, if carbon dioxide is allowed to bubble through the water, or if the animals are thrown into water previously charged with carbon dioxide, they at once become positively phototactic. The same results were obtained with other acids, as hydrochloric, oxalic and acetic, but boracic acid produced no such effect. Among the salts, he found that only those of ammonium could be compared in their effects with those of the acids.

Jackson[3] experimented with another amphipod, *Hyalella knickerbockeri*, which is also negatively phototactic, and obtained results similar to those of Loeb, except, that *Hyalellas* were made positive by boracic acid. Tartaric acid, however, produced no change in their reaction. With the salts and alkalies it was again found that there seemed to be no relation between the class of chemical used and the reactions.

Mast found that *Arenicola* larvæ which are normally positive, become negative in solutions containing various narcotics, acids, alkalies or neutral salts, and he claims that the sense of the reaction in these forms seems to be due to the general state of the

[1] Loeb, J., "Comparative Physiology of the Brain and Comparative Psychology," 1910.

[2] Loeb, J., "The Control of Heliotropic Reactions in Fresh-water Crustaceans, by Chemicals, especially CO_2" (University of California Publications), "Physiology," Vol. 2, p. 1, 1904.

[3] Jackson, H. H. T., "The Control of Phototactic Reactions in *Hyalella* by Chemicals," *The Journal of Comparative Neurology and Psychology*, Vol. 20, No. 3, pp. 259–263, 1910.

organism as a whole, rather than to the specific effect of acid or other solutions on the chemical state of some postulated substance.

My experiments on the effects of chemicals on phototaxis in *H. interpunctata* nymphs were performed in a dark room and the source of light was an incandescent bulb of seventy-five candle power. In each experiment twenty fresh specimens decidedly negative in their reaction to light were put in a long and narrow glass basin, three and a half by twenty-six inches, of about 2,000 c.c. capacity half filled with distilled water. The concentration of the solution was then slowly and gradually increased by adding constant small amounts of the given chemical at about ten or twelve minute intervals. A careful record was kept of the number of insects positive and the number negative at the different concentrations throughout the experiment. It was noticed that as the concentration of the solution was increased there was a gradual increase in the number that became positive. For example, in the experiment with hydrochloric acid at .01 per cent. solution, of the twenty specimens four became positive; at .02 per cent. eight were positive; at .03 per cent. fifteen were positive; and at .04 nineteen were positive. It was the same with the other chemicals employed. As a rule, specimens that once became positive remained positive throughout the experiment, and usually the insects that seemed to be the least annoyed by the light in fresh water were the first to manifest their positive phototaxis in the solution. In general the specimens that evinced their dislike for the light most obviously in fresh water were the ones that made the most frantic efforts to get at the light when they once became positive. However, the specimens as a rule were very persistent in their normal reaction, remaining negative until death occurred.

Of the different classes of chemicals employed the acids were the most effective in reversing the phototaxis of *H. interpunctata* nymphs. A large majority, and in some cases all of the twenty specimens became positive in the acid solutions. When CO_2 was bubbled through the water, again, a large percentage of the specimens became positive. The concentrations sufficient for this effect with the different acids used are,—hydrochloric .04 per

cent., tartaric .04 per cent., nitric .03 per cent., sulphuric .03 per cent., boracic .05 per cent., and acetic .07 per cent. Similar results were obtained with the salts, although the number of specimens which became positive was somewhat smaller. However, all the salts employed effected a reversion, and the concentrations necessary to do this are as follows: potassium iodide .3 per cent., potassium chloride .3 per cent., ammonium bromide .3 per cent., sodium chloride .3 per cent., sodium sulphite .06 per cent., and sodium sulphate .08 per cent. The alkalies were less effective, only about half of the specimens becoming positive in these solutions. In general all of the specimens became quite inactive, and when specimens which failed to manifest a positive response were placed in the end of the basin nearest to the light, they seldom made any effort to get away. All three of the alkalies, sodium hydroxide, potassium hydroxide, and ammonium hydroxide, which were employed, produced similar results at about the same concentration, which was .09 per cent. Alcohol, too, at a concentration of about .8 per cent. produced a reversal of the reaction in a large percentage of the specimens.

In their natural environment these insects live on the under side of stones and even in the aquaria I have never seen them on the upper surface. When a certain amount of any of the above mentioned chemicals was added to the water in the aquaria, the nymphs would soon crawl up on top. The acids, again, were particularly effective in this change, and although ordinary daylight was sufficient to bring the nymphs up, the results became more obvious when the aquaria were taken in the dark room and a strong light was suspended over them.

It gives me pleasure here to acknowledge my indebtedness to Prof. S. J. Holmes, for his help and kindly criticisms.

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A PRELIMINARY NOTE ON THE RELATION OF NORMAL LIVING CELLS TO THE EXISTING THEORIES OF THE HISTOGENESIS OF CONNECTIVE TISSUE.

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The connective issue of the adult is in the embryo derived from the mesenchyme. The same is true of certain other tissues, endothelium, muscle, and cartilage, bone and dentine, if these last be not included within the scope of the term connective tissue. It is in this narrower sense that the term connective tissue is herein used.

In considering the origin of connective tissue one has to take into account two distinct elements, cells and fibers. Of the cells there are at various stages in the embryonic mesenchyme three distinct types: (*a*) the wandering cells, viz., leucocytes, which are more directly concerned with the processes of hæmatopoiesis; (*b*) those cells which give rise to the true wandering connective tissue cells of the mature tissues, the mast cells, plasma cells, etc., whose history is more or less closely concerned with that of the embryonic leucocytes; (*c*) those typical connective tissue cells which are related to the fibers and which are commonly known as the "fixed" connective tissue cells. It is without doubt only the last type which is concerned with the origin of the connective tissue fibers, hence the present note deals only with this type of cell.

At least two types of fibers have also to be considered as arising in the connective tissue mesenchyme, the elastic and the collaginous. We are here concerned only with the latter type, for the reason that the elastic fibers arise at a later period; thus the origin of the connective tissue fibers concerns primarily the collaginous, or so-called "white fibers." It is unnecessary to distinguish at the early stage under consideration between such varieties of collaginous fibers as "reticulum" and "fibrog-

lia," for the former undoubtedly arises in exactly the same manner as the ordinary "white fibers," and of the histogenesis of the latter little or nothing is known if, indeed, it can be considered as an entity distinct from the other connective tissue fibers.

The numerous theories which have been proposed to account for the origin of the connective tissue fibers may be reduced to three chief divisions:

1. The embryonic connective tissue or mesenchymal cells become directly transformed by elongation into connective tissue fibers.

2. The fibers arise in the ground substance between the cells either by transformation of that ground substance or as a secretion from the adjacent cells.

3. The fibers arise within the cells either as distinct fibers (Schwann, 1839), as granules which fuse to form fibers (Spuler, 1896; Lavini, 1909), as an epicellular protoplasmic fibrous layer (Lwoff, 1889), or as an ectoplasm about the cell (Hansen, 1899) or forming the syncytium (Mall, 1902).

The theory of direct transformation by elongation of cells into fibers may well be abandoned and, except it be construed along the lines of the various theories of intracellular origin, it is worthy only of passing notice. Among other things the existence of an overwhelming number of fibers relative to the number of cells present in connective tissue argues against direct transformation, and modern microscopical methods are not able to detect the phases of such transformation either in normal growing tissues or in the process of wound repair. For several decades the results of observations have been in support of either the intercellular or the intracellular group of theories.

To Henle (1841) we owe the theory of the intercellular (extracellular) origin of connective tissue fibers, the fibers being supposed to arise from the intercellular ground substance, not directly from the cells. Latterly this theory has received but little support unless it be from the observations of Merkel (1895), who found that in the umbilical cord fibers appear first to arise in locations relatively remote from the cells. Later the cells wandered into these fibrous areas, and at a still later

period the fibers had so increased in numbers that they necessarily laid in relation to the cells.

The weak point in Merkel's deduction appears to be the disregard of the locomotive properties of mesenchymal or young connective tissue cells. If these cells, except for the incidents of mitosis, are stationary, then Merkel's observations would render conclusive evidence to support the theory of the extracellular origin of fibers. But that such cells possess at least a limited power of motion simulating the amœboid character has long been known. An adequate theory must account for the activity of these cells. Merkel's theory presupposes them to be stationary till fibers have appeared and only later to acquire amoeboid characters.

In searching for a tissue which would offer opportunity for the study of the activities of connective tissue cells in the living

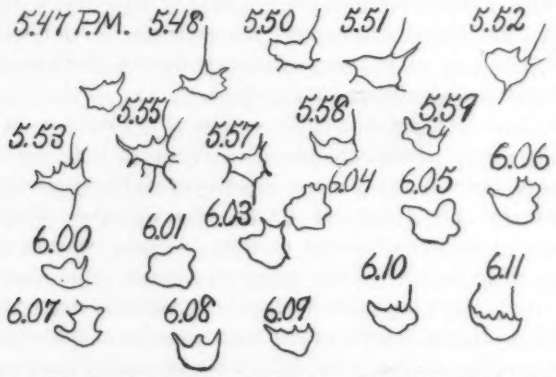


FIG. 1. Outlines of a stellate connective tissue cell in the caudal fin of a *Fundulus* embryo 4.5 mm. long (one day after hatching). The figures record the moment of completion of each drawing, the whole observation extending over a period of 24 minutes. The fish was afterward returned to water and swam for two days before being killed for further histological use. The cell observed corresponds in appearance with that shown by W. Flemming (*Arch. f. Anat.*, 1897, Fig. 9, Plate VI.) in the mesentery of a *Salamander* larva. It shows active amœboid motion and extensive changes of form. Its locomotion was not recorded, it was quite limited. × 800, camera lucida.

animal under normal conditions, I have found that the median fin of young fish embryos affords a subject for study which throws some light on Merkel's observations.

In a *Fundulus* embryo of 6 mm. total length—several days after hatching—I find in the median fin between the double layer of cutaneous epithelium the first signs of the dermal fin-rays. Blood-vessels have not entered, and between the fin-rays are few if any connective tissue fibers, certainly none in the distal portion of the fin. nvading the base of the fin is a mass of mesenchymal cells, mostly of the round cell type. Scattered through the fin are, here and there, isolated connective tissue cells in very limited numbers, distributed over an area representing the proximal one half or two thirds of the fin, and forming a sort of skirmish line, as it were, in advance of the army of round cells. These stellate cells can often be distinctly seen in the living animal. Later, connective tissue cells in abundance wander into the fin and fibers appear. But it cannot be said that the fibers appear in advance of the cells, for the "skirmish line" precedes the appearance of fibers, these advance cells becoming later intermingled with those of the subsequent invasion.

The advance cells, like all other stellate connective tissue cells in the fins of embryo fishes, I find to be actively amœboid, capable not only of motion but to some extent of locomotion. Since these cells are travelling through an area in which fibers are only just beginning to appear, the location of early fibers at points relatively distant from the cell in "fixed" tissue at once loses its significance and thus robs the theory of the extracellular origin of fibers of one of its strongest supports. My observations may be readily verified on any living, free-swimming *Fundulus* embryo within the first week after hatching.

Connective tissue fibers may be supposed to arise as a product of secretion of the mesenchymal cells, the extracellular deposition of fibers thus occurring under the influence of the cells. This is but a slight deviation from the strict extracellular origin presupposed by the theory of Henle and Kölliker. Such secretion must appear either in fluid form or as granules. In either case it is difficult to conceive why the secretion should take on a linear form if the cells remain stationary, and still more difficult if the cells are in motion, unless that motion be extremely slow and in one direction only.

By observation of the fins of embryo fish, chiefly *Fundulus*, I have been able to observe that the spindle cells of embryonic

connective tissue possess locomotion and move largely in one direction, but their motion and locomotion is extremely active, and so far as I can observe in the living animal they leave behind no trail of secretion, certainly no observable granules: still more important, the fibers appear to have been formed in great numbers prior to the appearance of the type of spindle cells in any considerable proportion relative to the other types of cells, round and stellate, already present in the primitive connective tissue. Hence I cannot conceive of the spindle cells as producing fibers by direct secretion.

But it is at the time of the predominance of stellate cells that the fibers make their first appearance in the fish's fin, as is likewise the case in the tissues studied by other observers. These stellate cells rapidly change their form, throwing out and retracting their processes in rapid succession, as can be seen in any living, free-swimming *Fundulus* embryo under 25 mm. total length. Fig. 1 shows the outline of such an early connective tissue cell in the caudal fin, its changes of form at intervals for a period of twenty-four minutes being accurately depicted with the aid of the camera lucida. It is impossible to both watch and draw all the changes in form occurring during this period, but sufficient are shown to demonstrate very active amœboid motion. By observing these cells in relation to a relatively fixed object, *e. g.*, a chromatophore, or a joint in an adjacent fin-ray, I have been able to detect in them considerable locomotion. I find that, unlike the spindle cells, the stellate cells appear to travel equally well in all directions. Neither following their locomotion nor in trail of a retracted process do I find any indication of secreted granules, nor of anything other than the most delicate, preformed fibers. One would suspect that if new fibers were being formed by a process of secretion they would appear within twenty-four minutes after such a cell or its process had occupied a given position in which fiber should reasonably be expected to be formed. Though I have repeated the experiment many times I have failed to observe such evidence of secretion, and these results do not appear to be consistent with a theory of direct secretion of fibers by the primitive connective tissue cells.

The intracellular theories of the origin of connective tissue fibers may be divided into two classes, those in which the fibers

are presumed to arise in the peripheral portion of the cell, as proposed by Lwoff (1889), or in a modified peripheral portion of the cellular or syncytial protoplasm, ectoplasm or exoplasm, as proposed by Hansen (1899) and afterwards by Mall (1902), and those in which the fibers are presumed to have a direct intracellular origin as observed by Spuler (1896) and later by Livini (1909).

The "circumcellular" origin of fibers proposed by Lwoff, since it must take into account the separation of fibers from the cells as well as their origin from intracellular granules, does not fundamentally differ from the later ectoplasmic modifications

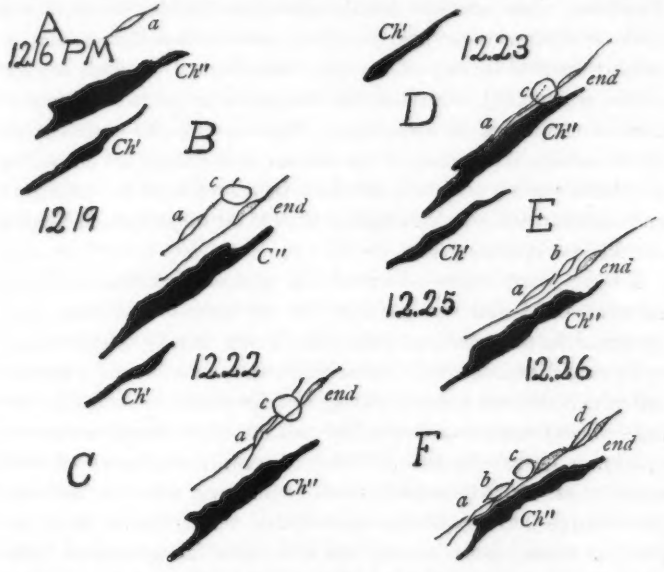


FIG. 2. A record of the locomotion of a spindle connective tissue cell, *a*, for a period of 10 minutes, adjacent chromatophores bordering on the fin-rays on either side being taken as the relatively fixed points. The cell was found to have covered a distance of about 50μ, a rate of 1μ in every 12 seconds. *a–d*, connective tissue cells; *c*, a round cell, the others spindle shaped; *Ch'*, *Ch''*, two chromatophores; *end*, endothelium of an afferent blood-vessel.

of Hansen and Mall. These later authors, basing their investigation on "fixed" material, presuppose a constant but gradual recession of the endoplasmic area with coincident changes in

the exoplasm in order to account for the transference of fibers from a position within the cell, in the ectoplasm, to an extracellular position. This hypothesis is entirely harmonious with the appearances to be observed in "fixed" tissue with the methods used. However, it is open to two objections, viz., the observations of Spuler, confirmed in a casual and not wholly satisfactory way by Livini, that fibers actually lie within the cells, and not always at the periphery, as Hansen's and Mall's theories presuppose, but even in direct contact with the nucleus; and, secondly, if the cells are in active amœboid motion, with locomotion as well, as is certainly the case in the fins of the embryo *Fundulus*, then one can hardly conceive that the more or less fixed syncytium described by Mall, whose observations can be readily verified by any of the usual histological methods applied to the tissue used, is sufficiently stationary to permit the retraction or shrinkage of endoplasm, thus leaving the endoplasmic fibrils outside the bounds of the cellular protoplasm, for according to Mall's theory true cells are lost, being replaced by nucleated endoplasmic areas anastomosing with their neighbors to form a continuous syncytial net.

I have many times observed the fins of *Fundulus* embryos between the lengths of 4.5 mm. (at the time of hatching) and 25 mm., the pectoral and caudal fins being usually selected, and in no single instance have I observed either a stellate or a spindle cell which did not exhibit changes of form and locomotion, the latter being sometimes very limited, at other times, as shown in Fig. 2, quite extensive. Hence the ultimate theory of connective tissue histogenesis, it would seem, must take into account the relative activity of the mesodermic cells. So far as I appreciate them, none of the theories thus far advanced fully meet all of the observed conditions. Further studies of both living and "fixed" tissue may further elucidate the true conditions involved in the histogenic process.

The fins of living fish embryos offer for the development of connective tissue a most desirable subject for study, for in the subcutaneous tissue between the dermal fin-rays one finds a definite connective tissue area, in which, with the exception of the somewhat related chromatophores, there is no other structure

to confuse the picture. This area is bordered on either side by the dermal fin-ray, beneath which an efferent blood-vessel descends, and alongside of which the return or afferent vessel ascends the fin; it is covered only by the pavement cells of the dermal epithelium, whose outlines are sharply defined, lying at a more superficial focal plane than the connective tissue. The chromatophores are easily recognized both by their color, black to yellow, and by their peculiar branching form, so that even those relatively or almost entirely devoid of color can be easily differentiated from the smaller colorless connective tissue cells. Moreover, the chromatophores are prone to lie in immediate relation to the fin-rays and to the blood-vessels.

In conclusion I desire to emphasize the opinion that in the study of histogenesis of connective tissue we must take into consideration the conditions surrounding the living cells and draw conclusions from "fixed" tissue only in the light of our knowledge of the living.

To furnish conclusive results living cells must be observed under normal conditions and not merely under those most unusual, or even pathological, conditions which surround the observation of growing tissues either in the mesentery studied by Flemming and others, open to the criticism of the presence of inflammatory changes, or in the tissue cultures by the more recent method of Harrison, Carrel and Burrows, which is surrounded by the necessity for the interpretation of results arising under entirely new surroundings and conditions as yet but little understood. Both in the mesentery and in tissue cultures movements of the connective tissue cells and even locomotion have been observed. I now add to these former results the record of the observation of motion and locomotion in cells under wholly normal conditions, in animals which underwent no operation other than in some instances the administration of chloretone, and which remained alive, active and normal for some time subsequent to the period of observation. For example, the particular fish which furnished the subject for Fig. 1 was returned to sea water and remained actively swimming for two days; he could have been kept much longer.

For the opportunity of pursuing this study I am indebted to the Marine Biological Laboratory at Woods Hole, Mass.

THE METHOD OF CELL DIVISION IN MONIEZIA.

C. M. CHILD.

Since the appearance of Richards's paper on the method of cell division in *Moniezia* (Richards, '11) Dr. Richards, in answer to a request of mine, has very kindly permitted me to examine some of his slides of early stages in the development of the ovary and of cleavage stages, with the privilege of publishing the results. The present paper consists of a brief statement of the results of my examination of these slides, together with some consideration of recent criticisms of my observations on amitosis. I take this opportunity of acknowledging my indebtedness to Dr. Richards and also of acknowledging an error of my own which though it does not directly concern the chief point at issue certainly requires correction. Dr. Richards is undoubtedly correct in his statement that the early cleavages in *Moniezia* are mitotic, either entirely, or to a much greater degree than my earlier statements would indicate. With this acknowledgment of my error the chief point of disagreement between Richards's positive observations, so far as they go, and my own is removed. The difference between us seems to me to rest now on Richards's failure to recognize, or to interpret as I have done, what the material actually shows.

The whole problem of amitosis seems to me somewhat unprofitable at present as a subject for investigation on a purely observational basis. Experimentation is necessary before we can reach any certain conclusions as to the significance of either mitosis or amitosis in the life of the cell. Moreover, cytological thought is at present dominated by morphological hypotheses, which are themselves based on direct observation rather than on experiment. So long as this remains the case all observations which do not accord with the current hypotheses will be explained away by one course of reasoning or another and brought into agreement with these hypotheses. So long then as our conclusions are based simply upon observation in fixed material and without any

attempt to control conditions in that material there will always be room for endless discussion and controversy. It seems to me highly improbable that cytology will make any very great real advances in this direction. But when cytologists shall seriously devote their energies to the problem of the physiology of the cell, a field of investigation that has been entered only here and there, and to the control of cellular phenomena, then I believe we shall see real progress, together with the consignment to oblivion of some of the hypotheses now most fondly cherished.

As regards *Moniezia*, however, I have at present nothing to offer except further observations, or rather my own interpretation of what I have seen in Richards's material as against his interpretation of what he has seen. Beyond the present paper I do not propose to enter into any further consideration of the problem of amitosis until I can do so on a different basis, as I hope to be able to do at some time in the future.

Thus far I have only attempted to defend my own observations and conclusions against what seemed to me unmerited criticism and skepticism based on insufficient data. Richards's paper on *Moniezia* is, however, a real reinvestigation of the subject and as such I can only welcome it and accord it the consideration which is due to any piece of scientific work, although I am compelled to criticize it in various points. The present paper includes a statement of the results of my observations on Richards's slides, together with some discussion of his arguments and conclusions and brief reference to criticisms offered by other authors and to the validity of cytological theory.

The slides contain exactly the same pictures as my own. There is no question here of differences of fixation or of staining. If amitosis occurs in my material then it occurs in Richards's in the same regions of the body and the same stages of development.

I. The Primordia of the Ovaries and Ducts.

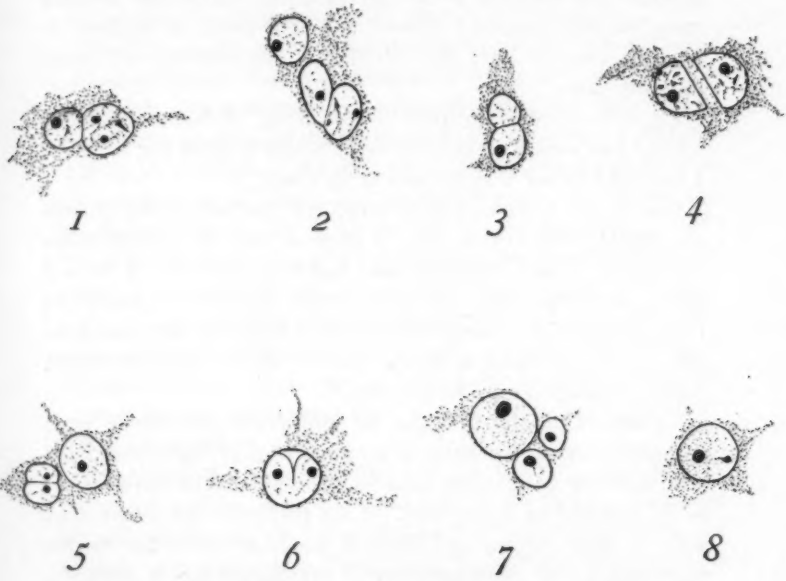
Figs. 1–8 are camera drawings from the primordia of the ovaries in Richards's slides. They were drawn from several slides with different methods of fixation and staining. In the figures I have attempted to reproduce what I have seen as

exactly as possible, though this is a somewhat difficult matter as regards the extranuclear phenomena. Godlewski ('09) has recently objected to my earlier figures on the ground that they were too highly schematized. This objection seems to me to be based largely on unfamiliarity with the material. In the early stages of the gonad visible cytoplasmic boundaries are often entirely lacking and the question as to whether cytoplasmic division follows nuclear division has no point, since it is quite impossible in most cases to distinguish cells. In later stages the germ cells do become distinct, but in those stages amitosis does not occur or occurs but rarely. In my earlier work I attempted to reproduce the essential features of the nuclei as exactly as possible. The only schematization consisted in not attempting to indicate an intranuclear achromatic structure except where such a structure was clearly visible and in leaving out various extranuclear details which did not bear upon the problem under consideration. In many of the nuclei the karyosome alone is stained and the rest of the nucleus appears almost or quite homogeneous; in other cases a more or less distinct structure of strands or an apparent reticulum does appear. In cases where I could not see such a structure distinctly I did not attempt to represent anything in the nucleus except the karyosome. It seems at least probable that the apparent achromatic nuclear structure is in many cases simply an artifact due to the action of the fixative. In the figures of the present paper I have represented a vague structure in all nuclei, but for most of the nuclei this must be regarded as little more than an attempt to show that something is there.

Richards's statement (p. 135) that the parenchyma is not a syncytium seems to me to require qualification. It may well be that it is not a continuous syncytium throughout the whole proglottid in later stages, but there cannot be the slightest doubt that several or many nuclei are often found imbedded in a more or less continuous mass of cytoplasm. This is true of the early stages of the gonads as well as of other parts. Where the nuclei are far apart they are usually, as Richards states, surrounded by more or less definite and distinct cytoplasmic masses, but this is certainly not true in cases where they lie close together.

Fig. 1 shows two nuclei in close contact, with the surfaces of contact flattened. If each nucleus surrounds itself with cytoplasm it is difficult to understand how a condition like Fig. 1 can arise except by division. Moreover, this is not the usual result of mitotic division in *Moniezia,* nuclei which arise by mitosis are separated by an appreciable distance when they form and are usually of equal size, which the two nuclei in the figure are not.

Fig. 2 shows a case which I am unable to interpret except as a direct division. The most careful focusing shows that the nuclei



are in the same plane and that the membrane separating them is not a strand of "linin" but a real membrane. Moreover, this membrane is apparently thinner and more delicate than other portions of the nuclear membrane.

In Fig. 3 two nuclei are shown which seem to be just completing division, much as in Fig. 1.

Fig. 4 shows two very distinctly hemispherical nuclei with flattened surfaces parallel and separated by a small amount of

cytoplasm which stains less deeply than other parts. They appear as if division had recently occurred and cytoplasm had begun to form on the adjoining surfaces. If it were not for certain preconceived ideas concerning the method of cell division, I think no one would hesitate to regard such a case as a very strong indication—I do not say proof—of direct division.

In Fig. 5 the apparent division of a small nucleus is shown. The membrane between the two parts is more delicate than the rest. The position of the two "nucleoli" is important. They are close to the separating membrane and directly opposite each other. This position is of very frequent occurrence in such cases, far too frequent I believe, to be the result of chance. It suggests very strongly that division of the nucleoli may occur in many cases.

Fig. 6 is a case of apparent "endogenous" division. Richards (p. 14) was unable to find anything of this kind in the material. I found a number of such cases in his slides.

Figs. 7 and 8 show cases in which the two halves of a nucleus are slightly different in color, a phenomenon of not infrequent occurrence, which Richards also failed to observe. I do not know, of course, that such a condition indicates division, but I regard it as highly suggestive in that it certainly indicates some difference in condition in the two parts of the nuclei and a certain degree of independence of each other.

Figures of this sort might be multiplied indefinitely from Richards's slides, but there is no reason for giving others here. My chief object is to show that exactly the same pictures appear in his material as in my own. I am perfectly well aware that none of these figures and likewise none of my earlier figures constitute a real demonstration of the occurrence of amitosis, for such a demonstration is impossible in fixed material.

Numerous similar pictures were found in the early stages of the genital ducts, the vitellaria and the parenchyma during the stages when increase in the number of nuclei is occurring in these regions. There is, however, no reason for giving figures from these organs, for on the basis of current cytological theory the point of chief importance is whether amitosis occurs in the germ cells. If its occurrence is admitted for these cells, most

cytologists would, I think, be willing to admit at once that similar phenomena in somatic cells doubtless indicate its occurrence there.

We may now consider the value of such pictures as evidence and the force of some of the criticisms made by Richards and others.

As regards the first point, I have observed pictures of this kind only in regions where the nuclei are increasing in number. Godlewski ('09) has suggested that I may have mistaken nuclear fusions for divisions. It would be strange indeed if nuclear fusion should occur only or chiefly in regions where the nuclei are undoubtedly increasing in number in some way.

Boveri's suggestion ('09) that the two nuclei in a cell represent the paternal and maternal chromatin refers primarily to the amphibian cells and perhaps he himself would not regard it as adequate to account for the appearances in *Moniezia*. Certainly there is no evidence of the separation of the two chromatins in mitotic division in *Moniezia*, even in cleavage. As regards the amphibian cells which I figured in an earlier paper (Child, '07*c*, Figs. 10 and 11) it should be repeated that the stages studied were not cleavage stages, but mostly larval stages, in which I could find no evidence, and I took especial care concerning this point, of a separation of paternal and maternal chromatin in mitotic division. I do not believe that this suggestion will account for the phenomena even in *Amblystoma* and it certainly will not in *Moniezia*.

As regards the possible increase of the nuclei by migration in particular regions, *e. g.*, the primordia of the gonads, Richards himself admits (p. 139) that the evidence for migration is not strong. Moreover, even if migration does occur in the formation of the gonads, there must nevertheless be a considerable primary increase in the parenchymal nuclei before the gonads are formed. Richards (pp. 138–9) finds the actual increase in parenchymal nuclei to be about equal to the increase in volume of the proglottids. Such an increase is, I believe, entirely inadequate to account on the basis of migration for the very large number of nuclei which appear in gonad formation.

Concerning the occurrence of mitosis Richards's actual ob-

servations are essentially identical with my own; he finds it but rarely, in fact he has not figured anywhere a case showing so many mitoses as my Fig. 8 (Child, '07*a*), in which seven cases of mitosis are shown. His argument concerning the ratio of resting to dividing cells in sections (pp. 139–40) concerns matters of which we really know almost nothing one way or the other; moreover, I am fully convinced that in many cases besides *Moniezia* where the infrequently observed mitoses have been supposed to account for all nuclear increase, numerous amitotic divisions actually occur. But besides all this, my conclusions concerning *Moniezia* are not based primarily on the frequency or infrequency of mitosis, but upon the actual observation of what I cannot regard as anything but amitosis. The infrequency or apparent absence of mitosis in many regions of *Moniezia* was what first led me to undertake a further investigation of the material and the remarkable infrequency of mitosis according to all observers in the neck region, the developing gonads and the genital ducts still seems to me to be highly suggestive, especially when we add to this fact the very frequent occurrence of what seems by all criteria at present available to be direct division.

Concerning periodicity in mitosis (Richards, '11, p. 140) the only positive observation which suggests anything of the sort is mine, which was cited in an earlier paper (Child, '10). This was a case of scolex, neck region and a few proglottids in which most of the nuclei were dividing mitotically. I have no means of knowing whether this is a normal occurrence, but if it is, it seems probable that this specimen is a young scolex, for the nuclei throughout the scolex are dividing as well as the others and these nuclei certainly do not take part in the formation of proglottids in later stages. I have considerable evidence for various forms which seems to me to indicate that many organisms or parts may start a period of nuclear multiplication by mitosis, while in later stages amitosis becomes increasingly frequent and if this scolex is normal it may be a case of that kind. It is quite possible, however, that it may be the result of certain special conditions of which we know nothing and not a normal stage of development in *Moniezia* at all.

It is certainly most strange, if mitosis occurs periodically or in waves as Richards suggests, that neither Richards nor Young nor I—except in the case above mentioned—have happened to observe any of these waves of mitosis in the numerous animals that we have sectioned. All the evidence which we have obtained indicates a remarkable infrequency and a scattered occurrence of mitosis.

But the argument based on mitosis is not direct evidence for or against the occurrence of amitosis. When we find the pictures which I have figured occurring frequently and confined to regions in which the nuclei are known positively to be increasing in number in some way, the direct evidence for the occurrence of amitosis seems to me to have far greater value than any indirect evidence based on the frequency or infrequency of mitosis.

On pages 149–50 Richards argues that in the primordium of the ovary and ducts the region of nuclear increase is at the periphery and states that it is here that mitosis is chiefly found. With the latter statement I agree, but I find it difficult to understand the grounds for his conclusion that nuclear increase is confined to the periphery. Certainly the increase in the number of nuclei at the periphery is not very great, while in the central region of the primordium the nuclei are very numerous, crowded close together. Moreover, these central nuclei are in early stages very much smaller than the peripheral. Unless there has been extensive migration from the periphery toward the center it is impossible to account for the great number of nuclei there except by proliferation. Richards has failed to find amitosis in this region, but I have found numerous cases of what I regard as amitosis in his slides. He maintains that the central nuclei are beginning differentiation (p. 150). I have been unable to observe any visible differentiation in this region at these stages and besides these crowded nuclei are on the average much smaller than any nuclei of the whole primordium after the visible differentiation has begun. In later stages, when, as I believe, proliferation becomes less rapid, these nuclei gradually enlarge again and become more widely separated and then visible differentiation begins. For these reasons I am unable to agree with

Richards that the region of greatest proliferation is the periphery of the primordium. Such a conclusion fails, it seems to me, to take account of all the observed facts. The great number, the very small size, the crowded condition of the nuclei, the relatively small amount or cytoplasm and the very frequent occurrence of apparent amitoses force me to maintain my original position, that the central region of the primordium is a region of more rapid proliferation than the peripheral. In connection with this point I have evidence from various species that in regions of more rapid proliferation amitosis is more frequent, while in regions of slower proliferation mitosis may be the chief or only method of division.

On p. 150 Richards states that "the method of cell multiplication in the female sex ducts of *Moniezia* cannot at the present time be positively stated." Here evidently the author has lost faith to some extent in his own argument, for the conditions in the ducts are not essentially different, so far as observation shows, from those in the primordia of the gonads themselves.

Of the possibility of observational error (Richards, '11, pp. 140, 141, etc.) no one can be more keenly aware than myself. I can only state again that I believe that I have used every precaution possible in direct observation of fixed material. I have often spent hours on a single case with the most careful focusing, changes of illumination with the aid of diaphragm and mirror and the alternating use of artificial light and sky light with and without color screen. Until Richards can show that he has taken at least equal precautions before concluding that direct division is absent, he lays himself open to the charge of error or superficial observation.

As regards the difficulty of observation in the small and often crowded nuclei of cestodes, a point which Richards emphasized in his earlier paper (Richards, '09), it is of course true that the nuclei are small and often crowded, but if one devotes sufficient time and care to the matter it is possible to find nuclei which show apparent direct division with almost diagrammatic clearness and I have seen many such, both in his material and in my own. In such cases I have not been able to convince myself by any means which I could devise that the appearance

of direct division was a deception or an artifact, or that it lay within the limits of observational error, as Richards asserts for all apparent cases of the sort observed by him.

It would be of interest to know how far Richards is familiar with the appearance of amitosis in forms and tissues where it is generally admitted to occur. I have spent considerable time familiarizing myself with such material and I find many cases of apparently dividing nuclei in *Moniezia* which seem to me to be identical in appearance with cases of division in such tissues.

And finally, Richards admits finding numerous "paired nuclei" and nuclei between which no layer of cytoplasm could be distinguished; it is of interest incidentally to note that his statements on this point concerning the ducts and vitellaria are much less cautious than those concerning the ovarian primordium itself. And although both he and I have observed mitosis in the ducts, he is willing to admit concerning them that there is "no certain evidence for amitosis and that for mitosis is, perhaps, insufficient to account for the growth which has taken place" (p. 150). And concerning the vitellaria he says, "if mitosis is not clearly proved as the sufficient cause of cell multiplication, amitosis is certainly less so." Concerning the ovarium primordium his statements are much more guarded. Here the influence of cytological hypotheses appears. Most authors are willing to admit the occurrence of amitosis in somatic cells, though they would regard as rank heresy the assertion that it occurs in the germ cells. Certainly *Moniezia* affords no basis for such a distinction and as regards many other forms we have not the slightest evidence that the germ cells in early stages are different in any way from somatic cells. The most that observation can tell us on this point is that in some forms they appear early, in others late.

As to what constitutes evidence for amitosis Richards does not seem to be entirely clear. As regards the vitellaria he says "but for amitosis there is only negative evidence" (p. 147), yet in the same paragraph he admits that "the arrangement of nuclei in pairs is, perhaps, more in evidence here and indented nuclei are somewhat more numerous." These observed facts represent conditions which we should expect to find present if

amitosis occurred and therefore constitute not negative but positive evidence of considerable value. They do not, of course, constitute a demonstration, but, as I have pointed out above, an actual demonstration or proof is impossible in fixed material. To my own mind, all the facts concerning which Richards and I agree, together with the cases which I regard as nuclear division, but which Richards has failed to find, constitute a practical, though not a logical demonstration of the occurrence of amitosis in *Moniezia*. On p. 127 Richards asks "whether the failure to find evidence of a certain process, using proper methods and exercising due diligence, is not positive evidence of the lack of that process?" Such a question can of course only be answered in the affirmative, but this does not constitute a refutation of my statement that Richards had presented only negative evidence for the occurrence of amitosis; it is of course merely the same statement in other words. And we are all aware that positive evidence of the absence of something must be of the strongest character before it can be regarded as refuting direct positive evidence of the presence of the same thing. Richards is unable to show with anything approaching certainty that mitosis is the only method of cell division in the ovarian primordium. I have presented positive evidence that amitosis occurs in addition to mitosis, though my evidence does not amount to an actual demonstration since that is impossible. I believe, however, that the evidence for the occurrence of amitosis in the ovarian primordia of *Moniezia* is almost if not quite as conclusive as that for its occurrence anywhere else in other species.

II. The Cleavage Stages.

As regards the cleavage of *Moniezia*, my examination of Dr. Richards's slides enbles me, as mentioned above, to correct the error into which I fell in maintaining that "cases of mitosis are rarely seen after the first cleavage, but amitosis is of frequent occurrence" (Child, '07*b*). Richards's observations as stated in his paper led me to believe that I must have been in error on this point and the examination of his slides removed any doubt which might have remained.

There is no question but that the earlier cleavages beyond the

first were almost entirely absent from the proglottids on which most of my study of cleavage was made and that my Figs. 21–26 (Child, '07*b*) are in reality sections of considerably later cleavage stages than I had supposed.

The passage of the eggs into the uterus in *Moniezia* undoubtedly occurs periodically and as the eggs are fertilized in the course of this passage it follows that in any single proglottid containing cleavage stages not all the stages will be present. In the proglottids on which I based my study of cleavage it happened that first cleavages were numerous, *i. e.*, a period of passage of eggs from the ovary to the uterus had occurred recently. But as a re-examination of these slides after my study of Richards's material, shows, the stages between the first and occasional second cleavages and much later stages were almost entirely absent. My figures of supposed early cleavages are in reality sections of eggs containing a considerable number of nuclei, but the plane of section happens to pass so as to show only or chiefly the large nuclei. That I could mistake these for early cleavages was due to the fact that I was much more interested in the condition of the nuclei than in the course of cleavage, and my observations on the latter point were only incidental and so led to a wrong conclusion. Thus my error on this point is explained, but I do not offer the explanation as an excuse.

Furthermore, Richards is entirely correct in his assertion that during the earlier cleavages the blastomeres possess distinct boundaries and the egg is not a syncytial mass.

On the other hand my examination of Richards's material has enabled me to discover what seems to me a very important point that he has failed to note. In his material the early cleavages are, as he says, mitotic and the blastomeres distinct. The cytoplasm in these stages stains rather deeply and uniformly and is not vacuolated to any great extent. At certain stages, however, which may vary in different eggs to some extent, but at which the egg consists of a considerable number of blastomeres it undergoes a marked change in appearance. The cytoplasm loses its tingibility to a large extent and becomes highly vacuolated and the distinct boundaries of the blastomeres disappear. Apparently these boundaries disappear gradually,

for in many cases some blastomeres remain more or less distinct while others have completely disappeared and in still other cases the whole mass of the egg appears to be a syncytium. In my earlier studies of the cleavage I interpreted these stages as stages in the appearance rather than the disappearance of the cell membranes.

In these and later stages I find, both in Richards's material and in my own, the most striking evidence for the occurrence of amitosis that I have seen anywhere in *Moniezia*. Here the nuclei are large and they are not crowded, consequently they can be studied with less difficulty and a larger number of good cases can be readily found.

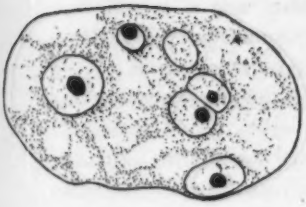
I give here a few figures drawn from Richards's slides to supplement my earlier figures.

In Fig. 9 five nuclei, one of them "double," are shown. No cell boundaries are visible and the cytoplasm is highly vacuolated and stains only slightly The two parts of the double nucleus are in immediate contact and the relation of the nucleoli to the dividing membrane and to each other which was mentioned above is seen.

Fig. 10 shows four nuclei, one of which appears to be dividing or to have recently divided. The two parts of this nucleus are of different size and are closely apposed.

Fig. 11 is an interesting case: the blastomere containing the double nucleus still retains its dense unvacuolated cytoplasm and to some extent its distinctness, though no actual cell membrane can be seen. The two parts of the nucleus which it contains are in immediate contact and flattened against each other. It is difficult to understand how they could attain such a position as the result of mitotic cleavage like that of the earlier stages. Moreover, in those earlier stages neither Richards nor I have found any evidence of the separation of paternal and maternal chromatin.

In Fig. 12 is shown a case of apparent division which seemed to me, as I examined it, particularly convincing. Here the two parts of the nucleus are of different size and a new nuclear membrane appears to be in process of formation between them. Its formation apparently began on one side and on that side the



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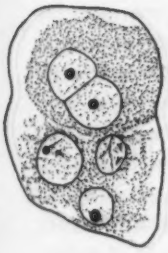

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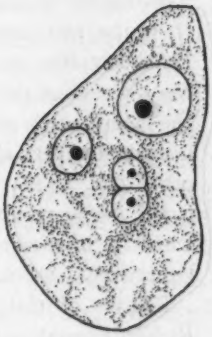

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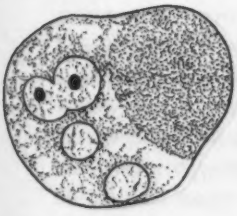

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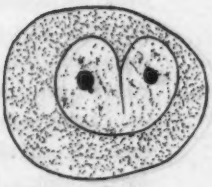

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nuclei have separated, while on the other side it is still very delicate and the nuclei are still closely connected. The dense mass in the upper part of the figure is the cytoplasm of a large blastomere which has not yet undergone the change in appearance; its nucleus is in another section.

Fig. 13 shows a section without any cell boundaries and with vacuolated cytoplasm in which one small nucleus is apparently dividing.

In Fig. 14 a constricted nucleus is shown. The cytoplasm of a large dense blastomere is also seen in the figure.

Fig. 15 shows another case which is to me very convincing. The double nucleus is in a mass of cytoplasm which is denser than the rest and still rather distinctly marked off from it. Evidently this represents a blastomere. Here, as in Fig. 12, the division of the nucleus apparently began from one side, for the membrane is much more distinct on the side where separation is most advanced and becomes more and more delicate toward the other side.

Fig. 16 represents a case of almost diagrammatic clearness in which a large nucleus is apparently dividing from one side. Other nuclei are in other sections of the egg.

Figures of this sort might be multiplied indefinitely from Richards's material as well as from my own. There is, I think, but little chance of observational error here, for the nuclei are relatively large and not crowded and in many cases almost diagrammatically distinct. If pictures of this sort are of any value as a criterion of the occurrence of amitosis, then the evidence is certainly very strong for these cleavage stages.

One question, however, must be raised here, viz., whether these are normal stages in development or degenerating embryos. To settle this point absolutely the study of later stages is necessary and thus far I have not been fortunate enough to obtain such stages. But in many proglottids practically every embryo shows this syncytial condition and if this indicates degeneration, then all, or almost all, the embryos of those proglottids are degenerating. It seems probable therefore that we have to do here with normal stages in the development.

III. Conclusion.

My examination of Richards's material has only confirmed me in my conclusion that direct division plays an important part in the developmental cycle of *Moniezia*, in the germ cells as well as in the soma. I believe I have at least shown clearly enough that this material contains something besides negative evidence for amitosis.

We are, I think, not yet acquainted with the nucleus as a dynamic system. We know something of it morphologically and almost all our hypotheses and theories concerning it are based on the morphological data obtained from fixed material.

But even when we consider only the results of direct observation it is difficult to understand how cytologists can continue as many do to ignore or minimize the importance of the rapidly increasing number of observations on the occurrence of direct division. Certainly the work of recent years on protozoa, to mention only this group, has brought to light many apparent facts which are not in agreement with current cytological theory. Where there is so much smoke it would seem that there must be some fire.

Moreover, when we regard the nucleus as a dynamic system rather than a morphological structure the necessity for assuming the individuality or continuity of the chromosomes disappears entirely. The reappearance of a definite and constant number of chromosomes in successive cell generations is essentially the same problem as the reappearance of five fingers on the hand in successive generations of man, or any other case of the inheritance of organs or parts in definite constant number yet we do not regard it as necessary to assume that these fingers or parts maintain their individuality at all stages of the life cycle. Instead of being the basis of heredity the chromosome is itself a problem in heredity. The theory of so-called chromosome individuality is a wholly unnecessary hypothesis and a heavy burden for cytology to carry, since it is not in accord with the facts and requires supplementary hypotheses at every point.

There is every reason to believe that the nucleus, like other dynamic systems, will behave differently under different internal and external conditions. What conceivable reason is there

to doubt that it can under certain conditions undergo fission or fragmentation and still retain the capacity to become a "whole" again? We know that the capacity for regulation is a very general characteristic of living things: are we justified in asserting without the strongest experimental evidence that the nucleus is wholly without this capacity?

If it were not for the fact that it makes no essential difference for the phenomena of life, including those of heredity, whether the chromosomes are continuous individuals or not this hypothesis would doubtless have been abandoned long ago. But since it is in a sense outside the field of scientific investigation, at least so long as present methods of cell study are in vogue, the chromosome theory has developed into a wonderful inverted pyramid of hypotheses in the construction of which the chromosome appears as a veritable *deus ex machina*.

The great need of cytology is a substitution of the experimental for the descriptive method: more experimentation and control and less inference from observation, a dynamic rather than a static point of view, rigorous proof rather than loose speculation; with such changes we may hope to gain some knowledge of the cell. The disadvantages of the present method are sufficiently manifest in the present paper.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
July, 1911.

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DROSOPHILA AMPELOPHILA LOEW BRED IN THE DARK FOR SIXTY-NINE GENERATIONS.[1]

FERNANDUS PAYNE.

In a short note (BIOL. BULL., '10) I stated that I had been breeding *Drosophila* in the dark for forty-nine generations or more than two years; that the darkness had produced no visible effect either in the color of the body or in the structure of the eye; but that there seemed to be a difference in their reactions to light, the ones bred in the dark reacting more slowly than those bred in the light. The evidence on which this last statement was based was meager. It was obtained by placing a number of flies in a clean vial and then revolving it so as to bring first one end and then the other toward the light. In this way it was demonstrated that the flies bred in the dark were noticeably much slower in their reactions than those bred in the light. These observations as far as they went were correct, but as we shall see they were not extensive enough and hence led to an erroneous conception.

I have just finished an experiment in which each fly was tested individually. This experiment was based upon four series of flies, 1,000 in each series: first (series no. 1), those bred in the dark for sixty-nine generations; second (series no. 2), those bred in the dark for sixty-four generations and then placed in the light for six generations; third (series no. 3), those bred only in the light; and fourth (series no. 4), a new strain from series no. 3 bred in the dark for five generations.

The apparatus for testing each fly consisted of a Welsbach lamp, a heat screen three inches thick and a glass tube one inch inside diameter and nine and three eighths inches long. The apparatus was arranged as shown in the diagram (Fig. 1), the end of the tube toward the light being ten and three fourths inches from it. The glass tube was perfectly clean on the inside and wrapped with black cloth on the outside. The flies were

[1] Contribution from the Zoölogical Laboratory of Indiana University, No. 120.

introduced into this tube by means of a shell vial three and one half inches in length and large enough to slide over the end of the glass tube. The end and about one half inch of the side of this vial were also covered with black cloth so when the vial was slipped over the tube, the tube admitted no light except at the end toward the lamp. By means of a stop watch I obtained the time it took each fly to travel the distance of nine and three eighths inches toward the light. Sometimes the flies did not react and in such cases they were counted out at the end of one minute. Records of these flies were also kept. This limit was placed at one minute, as experience showed that if they did not react within this time they might not react for five or ten

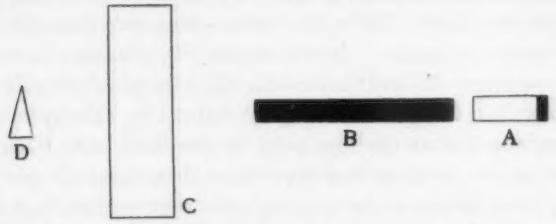

FIG. 1. Arrangement of the apparatus. *A*, the shell vial, three and one half nches in length; *B*, the glass tube, nine and three eighths inches long; *C*, the heat screen, three inches thick; *D*, the light, ten and three fourths inches from the end of the glass tube. The parts colored black were wrapped with black cloth.

minutes, or even longer. Extreme care was used to have external conditions as nearly uniform as possible. The temperature of the room at the time of testing varied between 74 and 78 degrees Fahr., but I could not see that a variation within this range made any difference. All flies were changed to perfectly clean vials and allowed to stand from ten to thirty minutes before testing, so they might become accustomed to a clean surface such as the inner surface of the tube into which they were introduced. The flies bred in the dark were placed in the light during this ten to thirty minutes in order to overcome any temporary effect of the darkness. All flies were tested at approximately the same age, from six to twenty-four hours after hatching. They were fed on banana which was permitted to reach about the same degree of fermentation before using.

With these precautions it was found that offspring of different parents within the same strain, those bred in the light as well as in the dark, vary considerably in their reactions. Further, this difference does not seem to be due to inheritance from the parents. To test this I selected five flies, each of which failed to pass through the tube in five minutes. The next day, I retested these same five flies and again they failed to react. This sluggishness was not due to poor development, as they looked strong and vigorous. These five flies were bred and their offspring tested. The average time of those which passed through the tube and the percentage which failed was approximately the same as for the series, indicating that something other than inheritance is the cause of this difference in reaction. I have taken two pairs, brothers and sisters, fed them on the same food, kept them side by side in the same room and yet their offspring showed a decided difference in their reactions to light. This fact has caused me a great deal of trouble and led to the erroneous conception stated in the previous note. In fact I can obtain the same result between the offspring of different parents in the same strain which I formerly stated was obtained by comparing flies bred in the dark with those bred in the light. Often this difference is so marked that it is noticeable before the flies are tested, those reacting least being more easily excited by handling. The only possible cause of this difference which I have to suggest is the food, as it may undergo different degrees of fermentation after it is placed in the vial. Another thing which leads me to believe that the food may be the underlying cause is that the last flies taken from any one vial are usually more sluggish than the first ones. Even with this difference in the reactions of the offspring of different parents, I believe the reactions of 1,000 individuals from many different parents gives a close approximation to the average for the strain.

The following table gives the average time of the flies in passing

1,000 Flies in Each Series.	Average Time of Flies which Passed through the Tube.	Percentage of Flies which Failed to Pass through the Tube in One Minute.
Series No. 1.....	13.9 seconds.	25.1 per cent.
Series No. 2.....	14.24 seconds.	23.6 per cent.
Series No. 3.....	17.62 seconds.	29.5 per cent.
Series No. 4.....	15.89 seconds.	23.2 per cent.

through the tube and the percentage which failed to pass through in one minute.

This table shows that the average time of the flies bred in the dark for sixty-nine generations (series no. 1) is least, 13.9 seconds, and the average of those bred only in the light (series no. 3) is greatest, 17.62 seconds. This is a difference of 2.72 seconds in favor of the flies bred in the dark. Likewise the percentage of flies which failed to pass through the tube in one minute is less (25.1 per cent.) in series no. 1 than in series no. 3 (29.5 per cent.). Series nos. 2 and 4 were run as controls and the average time is intermediate between the two extremes, while the percentage which failed to go through the tube is less than in series nos. 1 and 3. However, I do not believe the difference between any of the series great enough to be of any special significance and the only conclusion which can be drawn is that the darkness operating through a period of sixty-nine generations has produced no visible effect in the reactions of *Drosophila* to the light of a Welsbach burner. It should be pointed out, however, that the only cave condition present in the experiment is darkness; that it is possible and indeed probable that factors other than darkness (constant temperature and moisture) may play some part in the changes which have taken place in cave animals. If this be true the real test comes not in rearing animals in the darkness but in a true cave environment.

That the constancy of the environment may be a potent factor in the production of degeneration in cave animals is strengthened by the recent unpublished experiments of Tower. In a letter, August 2, 1911, Professor Tower makes the following statement:[1] "It has been my experience that there is hardly anything so injurious to breeding stock as a constant environment. It will produce degeneration, reduced activity, and not infrequently will result in actual elimination of the race." Also in the experiments of Calkins and others on protozoa, it seems that the constancy of the food supply and the constancy of the chemical make up of the medium in which they lived, brought about, at least a shorter cycle than occurs in a food supply and

[1] I wish to thank Professor Tower for the permission to use this statement before it has appeared in print.

medium which are changed at intervals as shown by Woodruff's experiment (*Archiv für Protistenkunde*, '11).

In caves conditions are practically uniform. Also in dark corners under stones and logs, conditions are more nearly uniform than in the open. Since it seems almost certain that forms which now inhabit caves once lived outside under stones and in dark corners and that they had varied in the direction of cave animals before they entered caves, is it not possible that the more nearly constant environment of the dark corners under stones and logs has started the degenerative changes which have been carried to their present condition in the more constant environment of the cave. To be sure all changes in cave animals are not degenerative. The tactile sense, for example, becomes highly developed, but no doubt a second causative factor enters here.

SOME PARASITES OF SIMULIUM LARVÆ AND THEIR EFFECTS ON THE DEVELOPMENT OF THE HOST.[1]

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During the spring of the present year (1911) while studying entomology at the Bussey Institution of Harvard University, I made numerous collections of *Simulium* larvæ, which are extremely abundant in the neighboring streams, with the intention of studying the development of the imaginal discs which are unusually well defined in this genus of diptera. As, however, I found that a large percentage of these interesting larvæ were heavily parasitized by two very different organisms, namely a worm and a protozoön, I turned my attention rather more directly to these and their effects on their hosts, during the few weeks intervening between my first discovery of the parasites and the pupation of the insects.

Before giving any details I wish to take this opportunity to offer my sincere thanks to Professor Wheeler, who by his kind suggestions and advise enabled me to bring together the following facts, which, though very incomplete in form, do not appear to have been recorded before. My thanks are also due to Professor Johannsen for naming the species of *Simulium* larvæ and to Professor T. H. Montgomery who identified the worm parasite as a species of *Mermis*.

LIFE HISTORY AND STRUCTURE OF SIMULIUM LARVÆ.

A brief summary of the structure, and mode of life, of the *Simulium* larvæ may not be out of place here.

If during the months of March to May one examines the rocks or vegetation in any swiftly flowing stream in the neighborhood of Forest Hills, Mass., especially where its bed causes a small cascade, one will, in all probability, observe a large, dark, gelatinous mass where the current is swiftest and the water

[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 45.)

shallowest. On closer examination the mass will be seen to consist of numerous curiously shaped larvæ standing upright on the rock, to which the hind end of the abdomen is firmly attached. These larvæ, the largest known North American species of which measure when mature some 12 mm., have the following characters: The soft-skinned body is more or less cylindrical, though the posterior third is somewhat swollen. The segments are poorly defined but the first three behind the head, namely, the thoracic segments, are usually distinctly marked off from the following abdominal segments.

The prothoracic segment bears a single leg (Plate I., Fig. 1) which is apparently two-jointed; the distal joint is small and retractile and terminates in a sucker which is armed with numerous hooks. No other segment bears any trace of legs, with the exception of the apical abdominal segment where the pair of anal prolegs of some other larvæ is represented by a powerful, armed disk-like sucker, by means of which the larva firmly attaches itself to rocks or vegetation when at rest. The skin is usually of a greenish gray color in immature larvæ, but gives place to a reddish brown as they mature.

The head is sub-cylindrical and is usually of a darker color than the rest of the body. Its chitinous integument is much denser and less elastic than that covering the remainder of the body. This results, during growth, in an ecdysis of the head capsule alone, being necessary more frequently than that of the general cuticular covering of the body. The new head capsule exposed after such an ecdysis is perfectly pigmentless in *Simulium hirtipes*, though dark spots soon form on its vertex (Plate I., Fig. 2, *a*, *b* and *c*), followed by a gradual infuscation of the whole surface. Individuals were observed in which the almost black head capsule was half removed, exposing the new pigmentless capsule, entirely independently of the body cuticle, the ecdysis of which was never observed, except at pupation.

On either side of the head are two black eye spots and on the anterior border are two lateral fan-like organs borne on elongated pedicels, which are used by the larva in procuring food. These fans consist of numerous curved rakes (Plate I., Fig. 3) bearing on one side long stiff cilia which, when the fan is expanded,

stretch from one rake to the next, thus forming a very fine strainer which allows water to pass through it, but retains any small organisms, such as the diatoms, on which the larva subsists. By means of a curious flicking motion these fans can be closed and their contents brushed into the mouth orifice. Here are situated two large glands (Plate IV., Figs. 9 and 10 (*d*)) on the dorsal side of the pharynx, whose function appears never to have been determined. They are covered with an apparently porous membrane which is clothed with short stout hairs. It would seem that they secrete some sticky material onto these hairs which removes the particles of food from the rakes when the latter are brought in contact with them.

It is usually stated that the fans are used to set up currents in the water and thus sweep food toward the mouth. My observations, however, lead me to believe that they act as a "strainer" and this is supported by the fact that, living as these larvæ do, in the swiftest currents, such movements would be useless.

The salivary glands are very large and secrete powerful silken threads which are used by the larvæ as anchor lines to hold them in an upright position no matter how strongly the current of water may flow.

Although the larvæ appear to be very sluggish, they can move about actively on the rocks by a looping motion similar to that of the geometrid larva. It is interesting to note the care with which these larvæ, when thus moving about, assure themselves that the adhesive disc of the prothoracic leg is firmly attached before relaxing the anal disc and *vice versâ*. Respiration is accomplished by means of retractile finger-like blood gills, normally three in number, which are situated on the dorsal surface of the last abdominal segment just above the anus. These gills, which can be distended at will by the insect, by means of blood pressure, are apparently inadequate for use in any but rapidly flowing water which contains plenty of oxygen, for if the larvæ be placed in a jar of still water very few will survive for more than eight hours, though life may be prolonged for two or three days by placing them in small numbers in Petri dishes containing only sufficient water to just cover them.

The form and size of the imaginal discs, or histoblasts, will here be described in detail as they are of especial interest in connection with the parasites.

The thoracic histoblasts are twelve in number and are very large and conspicuous in normal larvæ as they approach full growth (Plate I., Fig. 4).

The six discs to be found on each side of the thorax are the following:

1. Adult prothoracic leg histoblast—situated at the base of the larval prothoracic leg.

2. Adult mesothoracic leg histoblast—situated ventro-laterally on the mesothoracic segment.

3. Adult metathoracic leg histoblast—situated ventro-laterally on the metathoracic segment.

4. Adult wing histoblast—situated dorso-laterally on the mesothoracic segment This in the mature larva is by far the largest disc and it soon comes in contact with the mesothoracic leg disc.

5. Adult halteric histoblast This in the early stages is almost as large as the wing disc, but its growth and development are very slow and it soon disappears under the rapidly expanding wing disc.

6. Pupal respiratory tuft histoblast. This is situated on the prothorax and in its young stages has the appearance of being quite homologous with a prothoracic wing. It, however, soon begins to take on a definite form, and the comparatively stout tracheal tubes can be seen growing and lengthening beneath the transparent cuticle till they become coiled up as indicated in Plate I., Fig. 4.

It should be noted that this histoblast is not in the true sense of the word an "imaginal" disc since the respiratory filaments are exclusively pupal organs, and have no equivalent structure in any adult. Their similarity to a prothoracic wing destitute of the wing membrane is of interest. A few days before pupation this "pupal" histoblast begins to darken. Pigmentation commences at the apex of the folded tubes and slowly works backwards toward the base when the disc takes on the appearance shown in Plate III., Fig. 8*b*. Later, immediately prior to pu-

pation, the cuticle over this disc ruptures and liberates the fully formed and now functioning filaments.

The growth of these various histoblasts causes the thorax to swell considerably, thus giving the body the appearance of being constricted in the middle.

Internally there is comparatively little tissue. The abdomen contains the alimentary tract, and the much elongated salivary glands which lie normally in a ventro-lateral position with regard to the alimentary tract. The sexual organs are small and not easily found even in serial sections. The remaining portions of the body cavity are filled with blood plasma in which is suspended a quantity of fat body, mainly collected near the apex of the abdomen and causing this region to become slightly swollen. As the larvæ mature this fatty tissue very materially increases, and when dissected out, is of a stringy nature.

Methods.

Most of the larvæ were killed as soon as they were brought into the laboratory, but a few of the more heavily parasitized ones were kept alive in running water by covering the mouth of a jar with fine netting and introducing a piece of rubber pipe into the jar, through which tap water was run. In this manner specimens were kept alive for several days.

The following killing fluids were found to be the most satisfactory among several tried:

1. *Hot Water.*—Water was just brought to the boil when the larvæ were immersed and allowed to cool in the water. This method was most unsatisfactory from a histological point of view, but it had the advantage of leaving the skin as transparent as in its normal condition. It was also possible to dissect larvæ thus killed.

2. *Gilson's Fluid.*—This was used hot as described above and gave good results, but had the disadvantage of making staining with the hæmatoxylins difficult.

3. *Kahle's Fluid*, consisting of 30 parts water, 15 parts 96 per cent. alcohol, 6 parts formalin (40 per cent.), 1 part glacial acetic acid. This fluid has been recommended by W. Kahle (1908) and proved to be superior to Gilson's fluid both for

fixing and staining, and in the end was exclusively used. It was also used hot.

The chief advantage of both of these fluids was that, besides giving excellent histological preparation, they caused the histoblasts to turn milky white so that their position and form could be readily observed immediately after the specimens were killed.

Staining.—Heidenhain's iron hæmatoxylin combined with orange G was almost entirely used as it gave the best differentiation, though staining was rather slow. Replacing orange G with eosin accelerated staining but differentiation was less precise.

Mermis sp. Parasitizing Simulium Larvæ.

A number of larvæ were placed in a jar of water and left over night. The following morning I was surprised to see several white worms moving about at the bottom of the jar. It was evident that these had come out of the *Simulium* larvæ, so I went out to a ripple where the larvæ were particularly abundant and examined the colonies on several stones. I then noticed the large size of many individuals, and on examining these I found that many of them had a worm coiled up within the abdomen (Plate I., Fig. 5). When a quantity of material was brought into the laboratory it was found that these parasitized larvæ were much more sluggish than their healthy companions, which rapidly explored the jar in which they were confined, with their peculiar looping gait. The parasitized individuals, however, seemed much less concerned as to their new surroundings, and many of them were soon motionless, standing upright on the bottom and sides of the jar with their rakes expanded, ready to catch any food which might float their way. Here is a possible explanation of their larger size. Owing to their curious form of feeding it is evident that the more sluggish an individual is the more food it can obtain, and should it grow but a little larger than its companions, it will reach above their innumerable outstretched rakes, so that its food supply will be very materially increased.

It must not however be taken for granted that all parasitized larvæ were larger than their healthy companions, for some remained quite small, whereas many of the larger larvæ showed no

signs of worm parasites. As a general rule, however, the largest larvæ were found to contain one or more worms coiled up within the abdomen. One would naturally expect this rule not to be very constant, if, as conjectured, it is simply due to a more sluggish temperament and increased appetite on the part of the parasitized larvæ.

A case of a *Mermis* parasitizing ants was described by Professor Wheeler ('07) and here also he noticed a great increase in size of the host due to a greatly increased appetite during the larval stage.

A number of the worms were dissected out and sent to Professor T. H. Montgomery who pronounced them to belong to an undetermined species of *Mermis*.

Retardation of Development of the Histoblasts Due to Mermis.

On making a closer examination of the parasitized larvæ a far more remarkable effect than that of increased size was noticed, for it was found that the presence of *Mermis* parasites, no matter in what numbers, has a direct effect on the development of the histoblasts. In a normal larva of about 10–10.5 mm. which is the maximum length, and is attained immediately prior to the blackening of the respiratory filament histoblasts, the latter are quite large and owing to their white color readily visible to the naked eye; especially when the larva has been killed in Kahle's or Gilson's fluid (Plate II., Fig. 6). If a parasitized larva of the same, or greater, size be examined no trace of the histoblasts can be discovered with the naked eye, and can only be detected with difficulty under a dissecting lens. Under the low power of a compound microscope, however, they are seen to be represented by small white traces of the organs which should at this time be far advanced in development (Plate II., Fig. 7). A close examination fails to reveal any differentiation of these retarded histoblasts into the component parts of the adult, or pupal, organ.

The parasitized larva rarely develops beyond this stage, though I have observed specimens which were turning reddish brown, and contracting slightly as a healthy larva would, shortly before

maturation. In some cases, even, the rudimentary respiratory filament histoblast begins to darken in color.

I examined numerous specimens from the same stream at Forest Hills, and, with slight variations in intensity, they all showed this nearly complete suppression of the imaginal discs. Some weeks later I chanced to pass a small stream at Norwood, about seven miles from Forest Hills, and seeing *Simulium* larvæ present in small numbers, I took nine specimens from the rocks in order to see whether the species was identical with that common in our streams at Forest Hills. They proved to belong to the same species, namely, *Simulium hirtipes*, but I was much surprised to find that here also the *Mermis* parasite was much in evidence for of the nine specimens taken, six contained the worm. The effects however in these specimens were not so marked as in those found nearer home, for four of the six had turned brown and although the histoblasts were much smaller than is normally the case they were readily visible to the naked eye. Undoubtedly, however, maturation was impossible, for even should the larva manage to pupate it would soon die for want of oxygen, since the respiratory filaments were but half formed.

In a third locality, the Stonybrook Reservation near Forest Hills, a different species of larva was found. Professor Johannsen states that this larva is quite new to him and may be the undescribed larval stage of *S. bracteatum*, which occurs frequently in this State, but as I found only adults of *hirtipes* it was impossible to confirm this supposition. These larvæ were, however, also found to suffer from *Mermis* parasitism, but to a much less extent than those of *S. hirtipes*. The effects on the host were, however, as one would expect, identical with those on *hirtipes*, though the increased size was not so evident.

A large number of larvæ were measured, cut open and the worms removed. It is interesting here to note that even in fixed specimens where the fluid had caused the skin to become opaque, the presence or absence of worms could in all cases be determined with certainty by a glance at the histoblasts. The worm, it should be noted, did not cause the abdomen to swell up or become distorted to any appreciable degree.

A few selected results shown by the examination are appended:

	Length.	Color of Larva.	Histoblasts.	No of Worms.	Size of Worms.
1	10.75 mm.	Gray.	Minute.	12	Av. .75–1 cm.
2	11.50 mm.	Gray.	Minute.	1	2.75 cm.
3	11.0 mm.	Gray.	Minute.	1	3.00 cm.
4	11.0 mm.	Gray.	Minute.	3	1.3, 1.5, 1.5 cm.
5	10.5 mm.	Brownish.	Minute.	1	3.00 cm.
6	9.0 mm.	Gray.	Quite small.	3	.9, 1.3, 1.5 cm.
7	8.5 mm.	Brownish.	Large.	0	———
8	10.5 mm.	Gray.	Large.	0	———
9	8.0 mm.	Brownish.	Large.	0	———
10	7.0 mm.	Brownish.	Large.	0	———
11	8.5 mm.	Gray.	Large.	0	———
12	11.0 mm.	Gray.	Large.	0	———

From this table it will be seen that when there is but one worm in a host it attains a length of about 3 cm. during its parasitic life; that is, roughly about three times the length of the host itself, since the average length of a parasitized larva is 10.5–11 mm. It will also be seen that in one case as many as twelve worms were removed from a single host, and that in this case they all remained small, owing doubtless to insufficient food supply. In other instances, however, where several worms were present in one host it was noticed that one had attained almost to the normal size of 3 cm. whereas the remaining worm or worms were less than 1 cm. in length.

The larval measurements showed that parasitized larvæ average about 11 mm. (Plate III., Fig. 8*a*) whereas mature, and therefore somewhat contracted, larvæ, average some 8–8.5 mm. (Plate III., Fig. 8*b*), though a few are abnormally large.

Life History of the Worm.

As yet nothing is known of this except during the latter part of the life in its host. In all probability the eggs or young worms, are caught as they float down stream by the outspread larval rakes and are swept into the mouth, passing into the alimentary tract through the walls of which they bore their way till they enter the body cavity. It should however be noted that no scars or hypertrophy of the wall of the alimentary tract were noticed in several serial sections made of parasitized larvæ. The alternative hypothesis is that the larval worms bore into their hosts through the thin cuticular covering of the ab-

domen. That they should be present in the *Simulium* eggs is hardly possible, and this hypothesis can be with safety rejected.

Either of the former hypotheses offers a possible explanation for the variation in development of the histoblasts of parasitized larvæ taken from different streams, for in the case of those taken at Norwood where the discs were of a moderate size, the worms may not have entered the body cavity till the discs were somewhat developed. Then again where one worm has grown to nearly the normal size of 3 cm. whereas other worms in the same host have remained small, it is probable that the large worm entered the body cavity some time before the others.

Figure 9, Plate IV., which is a drawing of a median longitudinal section of a parasitized larva, shows the ventral position of the worm with regard to the alimentary tract, though it will be seen that the mesenteron has been somewhat pushed to one side by the coiled parasite. It will also be seen by comparing Fig. 9, Plate IV., with Fig. 11, Plate III., that the parasitized larva has much less fat body than that of a healthy individual at about the same stage of growth.

Another effect of the host is that apparently the sexual organs do not develop in parasitized larvæ. These are not easily seen in a healthy *Simulium* larva, as they are very small, but they can usually be found in a good series of sections; in sections of parasitized larvæ I have been entirely unable to locate them.

Otherwise the parasite appears to have no effect on the internal organs of the host, with the exception of displacing the spinning glands, the largest portions of which normally lie in the position later occupied by the parasite. Although they may be so displaced as to lie dorsad of the alimentary tract (Plate IV., Fig. 9, *b*) their functioning is in no way impaired, since these larvæ spin quite as many silken threads as do those which are uninfested.

The *Mermis* probably feeds directly on the blood plasma and fatty tissue of the host.

As the larva reaches maturity the contained worm becomes very restless, and if a living larva be placed under the low power of a microscope in a cell slide the movements of the worm can be easily observed. One thus watched for about half an hour was seen to explore the hypodermis of the host, evidently attempting

to find a place of exit. The head was kept constantly moving over the internal surface of the abdominal hypodermis and was even sometimes thrust far into the thoracic region. The movements appeared to cause the host great pain, especially when the thoracic region was visited. Finally after the entire worm had been several times twisted round in the body the head was pressed against the junction of the third and fourth abdominal segments and a hole was quickly made through which the worm slowly emerged. The worm, measuring 3 cm., took in all 27 minutes to disengage itself after puncturing the skin of its host. At first the operation was very slow and the constant writhing and turning of the host impeded rather than aided the movements of the parasite. When about 50 mm. were exposed the head was twisted around the body and the remainder of the worm was more rapidly forced out, finally becoming detached from the host in a tightly knotted mass which soon straightened out. The larva meanwhile rapidly shrank, and in about half an hour was dead. The worm apparently can leave the abdomen at almost any point, though the thin junction between two segments is usually chosen. It is probable that the death of most parasitized larvæ is directly due to the escape of the worm, which in all observed cases occurred some time before maturity.

On raising leaves in the bed of a stream a little below a large colony of *Simulium* larvæ it was found that they had under them several of these white worms. This year most of the worms had escaped by the beginning of May, and have since been lost sight of, though while examining a stone microscopically for a second brood of *Simulium* eggs, on May 29, one or two minute worms were seen, which may have been young *Mermis*. No *Simulium* eggs could be observed. At a still later date, August 3, a full grown, though dead, *Mermis* was found under a stone in the bed of the stream.

Percentage of Larvæ Infested.

Several leaves covered with larvæ were taken from a stream in Forest Hills for the purpose of determining the percentage of parasitism.

These larvæ, which represented the species *Simulium hirtipes*,

gave the following results: Number of larvæ present 174; number of parasitized larvæ 41. This means that in this particular locality about 23.5 per cent. of the larvæ would be unable to mature on account of the *Mermis* parasite.

The species living in the Stony Brook Reservation was found to be parasitized only to the extent of some 3–4 per cent. A few *S. hirtipes* were also present in the same stream and were parasitized only to the same extent, so it is probable that this smaller attack was not due to the host's belonging to a different species.

Cause of the Retardation of the Histoblasts.

In order to arrive at some definite conclusion as to why the histoblasts of a parasitized larva should not develop normally, it will be of advantage to review briefly some of the theories which have been advanced to explain the normal development of these discs, and at the same time to consider the facts which have been brought to light by the study of several cases now on record in which the discs have developed with abnormal rapidity, thus producing larvæ which possess characters that normally do not make their appearance until the pupal or adult condition is reached. This abnormal condition has been termed "prothetely" by Kolbe (1903).[1] For the abnormally retarded condition as produced in the *Simulium* larvæ by the presence of *Mermis* I should like to suggest the word "metathetely."[2]

The earliest report of prothetely was made by Heymons ('96). In this case larvæ of *Tenebrio molitor* L. were found which, when mature, proved to be abnormally developed as follows:

1. The meso- and meta-thorax possessed expanded lateral portions of the tergites, which resembled the wing-pads of the pupa, though they were not folded under the body as in the latter, but were directed backwards.

2. The antennæ had additional incompletely segmented joints, thus approaching the 11-jointed adult condition.

3. The abdominal tergites were modified so that they resembled the tergites of the pupal abdomen.

These larvæ were raised in the mealworm cultures of the

[1] Προθεῖν, to run before, and τέλος, the completion.

[2] Μεταθεῖν, to run behind, and τέλος, the completion.

Berlin Zoölogical Institute, but no statement is made as to whether conditions during development were quite normal. Heymons concludes his paper by suggesting that the abnormal development described above is due entirely to an accelerated development of the histoblasts, but makes no suggestions as to the cause of the acceleration.

In 1903 Kolbe reported and figured an interesting case of prothetely in the larva of *Dendrolimus pini* L. He received the larva when in its fourth moult, at which stage it had the following characters:

1. The larval antennæ were replaced by elongate antennæ showing simple primary division into about seven segments.

2. The larval thoracic legs were replaced by three pairs of jointed legs possessing all the adult parts, namely, coxæ, trochanters, femora, tibiæ and tarsi.

3. The mouth parts were modified.

Kolbe points out that all these organs were in an immature adult, or true *pupal* form, thus showing, it would seem, that the development was quite normal, but simply accelerated. Winneguth succeeded in breeding from a similar larva an adult which hatched as a small male that was apparently quite normal except for its dwarf size. These abnormal larvæ were produced from an artificially hatched generation kept indoors and from parents which did not hibernate.

Hagen ('72) gives an account of silkworm larvæ obtaining wings before pupation, which condition was accompanied by other abnormalities as follows: The head was small and had two small facetted eyes, and the thorax became modified but the abdomen remained in the normal condition of a larva in the fourth moult. The fore wings were long and narrow and rather more gray than usual, while the hind wings were short and narrow. As this anomaly occurred frequently and was therefore liable to be of economic importance, its causes were investigated by Majoli who came to the conclusion that it was due to the larvæ being kept at a temperature above the normal.

A case similar to that of Heymons was described by Riley ('08) from another coleopterous larva, *Dendroides canadensis*, which was bred by a student at Cornell University.

It will be seen from these cases that in every instance they occurred in artificially reared larvæ, and it is probable that the prothetely was due to an increased temperature, perhaps with the aid of abundant food, in some way hurrying on the development of the histoblasts.

This fact suggests that the histoblasts may be caused to develop, though at a much slower rate than the larval organs until pupation, by some enzyme secreted by the insect and that they can develop only as fast as this stimulant is formed. Its supply thus acts as a regulator. An increased temperature is in most cases advantageous to enzyme action and in this case it is probable that it either causes more of the enzyme to be secreted or stimulates the action of the amount already available.

Dewitz ('05), after numerous experiments on retardation and acceleration of development and pupation arrived at the following conclusions: Development and pupation are caused by enzymes which are not very evident in the early larval stages. They, however, increase with the growth of the larva until its pupation, at which period they are at their maximum strength; they then begin to diminish, till at the end of the pupal period their action entirely ceases. He also states that the enzyme action can be hindered by the presence of another body; and that by obtaining an enzyme of an increased strength before pupation development can be abnormally accelerated.

It is evident that the cells of the histoblasts are caused to develop by a different stimulus from that which causes the development of the larval organs, for in the former case development is very slow during the larval stage, when the latter organs are developing very rapidly, and it is only when these have reached their limit of development at pupation, that the adult organs begin to develop with any rapidity.

This suggests that there may be in the insect body two sets of enzymes which one may term "larval" enzymes and "imaginal" enzymes. These are sufficiently different so that conditions which cause the acceleration or retardation of one of them need not necessarily have any effect on the other. If this be so, one has a probable explanation of prothetely and also, as I shall attempt to show in the following paragraphs, of metathetely.

At first sight one would suppose that the retardation in development of the histoblasts in parasitized *Simulium* larvæ is simply due to a lack of proper nourishment for these organs, since the larva, besides having to supply the requirements of its own developing larval tissues, has also to supply the demands of its fast-growing parasite. This may be true to a certain extent, but later observations, when another parasite is also present, indicate that there must be some other more potent factor whch accounts for this inhibition. This second parasite is a Sporozoön which, owing to the vast numbers in which it occurs in a single host, is far more bulky than the worm and must, one would imagine, make far greater demands on the resources of its host. In this case, however, the histoblasts are usually unaffected in size, though in many cases they are distinctly smaller than normal. Two individuals, however, were seen in which the histoblasts were minute. On dissection it was found that, in each case, a small worm measuring only some 7 mm. was living embedded in the mass of spores, and it was evident that this minute worm was responsible for the retarded condition of the discs, even though it had evidently absorbed very little nourishment. One must, therefore, in all probability look to some *toxin* secretèd by the worm as the cause of the inhibition of development in the discs.

The researches of Verson and Bolle, as quoted by Fischer ('06), proved in the case of lepidopterous larvæ that in their early stages their body fluid is alkaline and that this alkalinity decreases as the larva matures. This would suggest that an alkaline condition encourages the growth of larval tissues whereas acidity, or the absence of alkalinity, permits of the development of the adult organs. Hence the histoblasts would develop but slowly till the larvæ are nearing maturity when the decreased alkalinity of the blood allows the "adult" enzymes to stimulate the cells of these discs to rapid division. I have been unable to find any account of the excretions of *Mermis* or of closely related Nemathelminthes, but should they be proved to have an alkaline reaction the probability of the above contention would be very greatly strengthened, for here we should have a case of the alkalinity of the system being maintained in maturing larvæ, and thus preventing the normal, though slow, development of the

adult organs, without affecting the larval organs to any great extent, except in so far as they are kept well supplied with alkaline fluids and are thus capable of developing to their utmost extent. This would account for the somewhat larger size of parasitized individuals.

It may be, however, that the *Mermis* does not actually secrete an alkaline substance, but brings about an increased alkalinity in the body of its host by absorbing whatever acids are formed in it. The probability of this being the true condition is increased by the fact that closely related worms live in acid media, such as vinegar or sour paste (*Anguillula aceti* Chrbg.), which would point to the fact that the worm requires, and absorbs, acids during its development. In either case the effect on the host would be the same in that there is a reduction in the activity of the acids which appears to be essential to the development of the histoblasts. Whether this is the true explanation or not, it is certain that the presence of the worm does have a direct inhibitory effect upon the development of the imaginal organs, but does not have a similar effect on the larval tissues.

A suppression of pupal and adult organs will naturally be of advantage to the parasite for two reasons:

1. Nourishment is not required for building up these tissues and therefore more will be available in the body cavity of the host.

2. The maturity of the host will be deferred thus giving the parasite a longer life, should it require extra time for development. In most of the observed cases, however, the worm killed its host, by emerging before or at about the same time that the uninfested larvæ were pupating.

A similar though less marked case of metathetely due to parasitism by *Mermis* has been described and figured by Mrázek ('08) in the queens of a European ant (*Lasius alienus*). In this case the parasitized larvæ matured and produced adult ants which were normal in all external characters with the exception of a great reduction in the size of the wings. Through the kindness of Professor Wheeler I have been able to examine some similarly parasitized specimens of a closely related American species (*Lasius neoniger*) in order to see whether the development of the

legs had been in any way affected but a careful comparison of the measurements of the legs of the parasitized ants with those of healthy specimens failed to reveal any inhibition of their development on account of the *Mermis*, although the wings, which in normal ants measured some 10–11 mm. in length were reduced in the parasitized individuals to 6–6.5 mm.

In the case of *Simulium* larvæ, as before stated, the development of the legs also is inhibited by the presence of *Mermis*, though comparative measurements of the wings and leg histoblasts in healthy and parasitized larvæ show that whereas in the former case the wing histoblast covers about four times the area covered by that of the mesothoracic leg, in parasitized larvæ these histoblasts bear a relation to each other in size of about 2.5 : 1, showing that the wing histoblast suffered a greater inhibition in development than did that of the leg.

A further interesting case of *Mermis* parasitizing ant larvæ was described by Wheeler ('10), in which case the worker larvæ of *Pheidole commutata* were parasitized, and resulted in the adults of such larvæ not only possessing all the normal "worker" characters perfectly developed, but owing to excess of feeding on account of their constant hunger these "worker" larvæ developed, when mature, characters such as the ocelli which are normally only found in the sexual ants.

A Sporozoön Parasite of Simulium Larvæ.

While dissecting out worms from a batch of larva taken on March 30, I chanced to cut open one larva which from the whiteness of the abdomen I took to contain a worm, but was much surprised to find that the body cavity was closely packed with a white substance which had the appearance of cotton wool. A little of this substance, however, when smeared on a slide was seen to be composed of countless organisms as illustrated in Plate V., Fig. 14. My first impression, very naturally, was that these were spermatozoa, which they resemble very closely in general outline. It was, however, very difficult to imagine to what possible organism these spermatozoa could belong. The larva itself could surely not produce them; but if not the larva what then? The only explanation seemed to be that they were

formed by one of the worms, which could not then be *Mermis* but must be a new form closely related to *Allantonema* or *Sphærularia*, in the females of which the uterus becomes protruded and is finally many times the size of the original worm.

I naturally visited the stream in order to obtain more specimens suffering from this disease, only to find that a quantity of oil which had been flowing down the stream for some days past had succeeded in killing off all the larvæ in that neighborhood. It was therefore necessary to find a place further up the stream, above the contamination, where the larvæ were living. About half a mile's walk led to such a place, situated under a stone arch, in which the larvæ were present literally in thousands. They formed great masses covering the whole surface of the rocks. An examination of a few rocks soon showed that the *Mermis* was very plentiful here, but it was some time before I found specimens with the curious white abdomens for which I was searching. I chanced, however, to pull up a piece of water weed that was floating in a swiftly running swirl and here I found quite a number of individuals, some showing immense abdominal swellings due to the parasite. On examining a few small specimens I found that the parasite was more numerous among them than among the larger individuals; a closer examination of the rocks showed that these small parasitized individuals were quite commonly scattered among the larger healthy ones. A quantity of material was taken back to the laboratory to be studied on the same lines as that adopted for the *Mermis* parasite.

External Effects on the Larva.

The first effect noticed in badly infested larvæ is the immensely distended abdomen. In some cases as in that illustrated in Plate V., Fig. 12, the apex of this region of the body was three to four times its normal size. Unlike the *Mermis*, this parasite is not confined to the ventral portion of the body but entirely surrounds the caudal extremity of the mesenteron, and small detached colonies were not infrequent in the thoracic region. The Malpighian tubules, however, can usually be seen on the surface of the mass and are plainly visible through the tightly stretched skin, which is quite transparent in places and appears to be on the point of bursting.

The length of the larvæ next claims attention. No larva containing this parasite was found to be exceptionally large; whereas, as before mentioned, many were extremely small, measuring some 2.75 or 3.5 mm. at a time when all normal larvæ measured some 9 mm. or more. This may possibly require a similar, though opposite, explanation to that suggested on page 281 to account for the large size of the individuals affected by the *Mermis*.

In confinement these small specimens were extremely restless, they were continually twisting about and moving over the sides and bottom of the jar with a peculiar jerky movement. The larger, and more heavily parasitized, larvæ on the other hand were more sluggish though not more so than the healthy larvæ.

The effect of these parasites on the histoblasts is very hard to state with any certainty. Fig. 13 is an illustration, made with a camera lucida, of the histoblasts of a nearly mature individual. In this case the parasite was confined to the abdomen. It will be seen that the discs are but half developed (compare with Fig. 6). In other cases, however, the development of the discs was not, so far as could be seen, affected in any way till pigmentation of the respiratory filaments commenced. Then it was noticed that the entire histoblast only turned a slate gray color instead of blackening at the apex of the filaments and finally over the complete disc. In other cases, however, the discs entirely blackened in a perfectly normal manner.

When colonies of parasites are located in the thorax, as is not infrequently the case, the histoblasts are materially decreased in size, while in the very small specimens development appeared to have been arrested. This is hardly surprising. My general conclusions were that the parasite affected the histoblasts but little, though the larvæ rarely advanced so far toward maturity that the respiratory filaments became pigmented. It should be noted that the power of spinning silk was in no way affected by the presence of this parasite.

Nature of the Parasite.

While visiting other localities I made numerous observations on the larvæ to be found in the streams, and was much surprised to find that species of the parasite were extremely common and

that in almost every brook visited some of these conspicuously distorted individuals were to be found. On making microscopical examinations I observed that the organisms taken from different localities varied very much in form and apparently represented different species. A list of these different forms and their hosts is appended:

1. Ovoid bodies bearing a "flagellum"-like organ varying in length from about that of the "body" to three times its length (Plate V., Fig. 14). Host: *Simulium hirtipes*. Habitat: Forest Hills and Blue Hills, Mass.

2. Bi-annulated ovoid bodies bearing a "flagellum," which is never much longer than the "body" (Plate V., Fig. 15). Host: *Simulium* species undescribed. Habitat: Stony Brook Reservation, Mass.

3. Ovoid bodies having the "flagellum" replaced by a transparent flattened disc (Plate V., Fig. 16). Host: *Simulium* species undescribed. Habitat: Stony Brook Reservation, Mass.

4. Simple ovoid bodies destitute of all appendages (Plate V., Fig. 17). Host: *Simulium* species undescribed. Habitat: Stony Brook Reservation and Blue Hills, Mass.

The following characters were common to all of these organisms:

1. They were all similar in size.

2. Under the highest power of the microscope they had a faint olive greenish tinge.

When in a fresh condition absolutely no internal details were visible and in specimens which had been killed, fixed and stained very little more could be seen. In many of the first or "spermatozoid" type a darkened central area indicated the presence of a very large nucleus, while a smaller dark spot just before the base of the flagellum might be taken for the nucleolus.

Many of the "simple ovoid" type, although but slightly staining, showed a great shrinkage of the internal substance; otherwise nothing could be seen in them. The specimens with a flattened disc, and those bearing two annuli and a flagellum, also showed practically no differentiation. Methyl green and various hæmatoxylin combinations were tried without success.

Besides these various forms, no two of which were observed

to occur in the same individual, another form of cell (Plate V., Fig. 18) was found in much smaller numbers, but possibly in some way connected with them, for it was seen in association with each form, but was not found in healthy larvæ. This cell, which varied much in diameter from but little more than the numerous "spores" to three or four times the length of their longest axis, was apparently globular in form, and in a fresh state was very transparent and could only be discerned with difficulty. The following characters, however, were seen. The substance of the cell was finely granular, and often contained a number of large transparent globules. When fixed and stained the only differentiation was that of a large dark mass, apparently the nucleus (Plate V., Figs. 18, *a* and *b*). In some cases these cells seemed to be dividing (Plate V., Fig. 18, *c*).

In the face of all these diverse types of cell, which live in precisely the same way, it is evident that the forms first found are not spermatozoa, and further the fact that the flagellum is replaced in one "species" by a flattened disc eliminates the possibility of their being *Flagellates*. It is therefore probable that one must look to the *Microsporidia* among the *Sporozoa* as the group to which these bodies belong. It is further noticed that many of the forms are very similar to those met with in the genus *Glugea* to which the well known *pébrine* disease (*G. bombycis*) of silk-worms belongs. One must therefore conclude that it is a pebrine-like disease, which is killing off a high percentage of the Simuliid larvæ in the neighborhood of Boston, though on account of the vast difference in structure and mode of life of the two hosts the life history of the parasite in the *Simulium* larvæ is very unlike that described by Pasteur ('70) and Stempell ('09) in their work on the disease in silk-worms.

Life History of the Parasite in Its Host.

As in the case of *Mermis*, I know very little about this, since I did not discover it till the final stages of the larva were being approached, and in every instance but one it was apparently in exactly the same condition, namely, the "spores" were all formed and were simply awaiting the death of their host and its resulting decomposition to escape into the water.

The one exception, however, was that of a larva found on April 17. It was one of the first discovered, in the abdomen of which could be seen, with a dissecting lens, large white bodies floating in the blood plasma. On dissection these bodies proved to be rounded masses of flagellate spores, a few of which had become disengaged and were apparently moving about by their own impetus in the blood plasma. As, however, I have never on any subsequent occasion observed such a movement among these spores I am inclined to believe that this was simply a Brownian movement, which I have distinctly recognized in later examinations. Though I searched carefully for other larvæ with parasites in a similar stage I was unsuccessful.

In serial sections of parasitized larvæ similar effects to those exhibited when *Mermis* is present, are noticed in that the fat-body is much reduced (Plate IV., Fig. 10) and the sexual organs could not be detected, whereas the spinning glands and musculature apparently remained unaffected. The parasite probably enters the host through the alimentary tract for in all cases of infected larvæ it was found that the mesenteron was distorted in one or more places showing distinct hypertrophy as if the cells had been badly irritated but had healed over again often permitting the spores to pass through into the body cavity (Plate IV., Fig. 10, *e*). In one case a small colony of "spores" was found inside the alimentary tract, but it is quite possible that these had been recently taken in with the water, as parasitized larvæ were constantly dying in the colony, and any material, floating in the water such as liberated "spores," would be caught by the cephalic fans of still living larvæ.

In all recorded cases of Microsporidia parasites it has been noticed that the "spores" live, at least during their early stages, inside some body tissue of the host. An example of this is to be found in pébrine of silk-worm larvæ, in which case the spinning glands are the main seat of attack. It must be borne in mind, however, that an attack upon these organs in the larva now under consideration would, in all probability, result in the early death of the host, for it is only by means of the much strengthened silk threads that the larva is enabled to maintain in rapid streams the perpendicular position which is essential to obtaining food,

while at the same time the larva has to depend to a large extent on these threads for retaining any foothold on the rocks, for when moving its position, the adhesive disc of the proleg frequently loses its grip and the larva is washed clear of the rock. Very rarely, however, the anchoring threads, which are always present, break so that the larva is able slowly to draw itself once more to its support. Sections of the *Simulium* larva disclose the fact that there are very few other tissues in the body. The muscular system is much reduced, and the only tissues available for attack without rapidly killing the host are the fat-body and the sexual organs, and I am under the impression that it is the latter which are usually the original seat of attack. In frontal sections of a very young larva taken during March, only one testis could be found, but on the other side of the body a small mass of minute cells, taken at the time for small œnocytes, was situated a little back of the normal position of the missing testis. The cells are too small to show any structures but it is possible that this is a diseased testis to which the spores made their way as soon as they entered the body cavity. Later sections also show traces of a very thin membrane investing the mass of "spores."

I kept a large number of diseased larvæ in running water, hoping to see some of them pupate, but in every case they died, and soon liberated the spores, whereas many of the healthy larvæ, which were approaching maturity, pupated in captivity. It is thus evident that heavily parasitized larvæ never pupate, but die in the stream, liberating their countless spores in the water. What happens to these spores I have been unable to ascertain. Larvæ were allowed to die in distilled water and the liberated organisms were examined at intervals, but though they strongly resisted decomposition they never showed signs of movement or altered condition. Sections of pupæ and adults obtained from badly infested localities failed to reveal any cases of the disease being carried beyond the larval stage. It is therefore probable that the disease is not hereditary as is the case with pébrine, and as before stated it was seen that the presence of this parasite, in every case examined, apparently resulted in castration of the host. It is, however, possible that among the vast numbers of larvæ a few were only slightly parasitized and did not have their

sexual organs entirely destroyed. Such individuals should be capable of pupation and might in that way carry the disease over as in the case of pébrine. An examination of several hundreds of larvæ did not reveal one in which such a condition was possible.

Were there overlapping generations of larvæ the maintenance of the parasite could be more easily understood, but the spring brood pupated and hatched during the first half of May, and there are not at the time of writing any signs of more eggs being deposited on the rocks. It thus seems that there must be a secondary host in which this parasite passes the summer.[1]

The Percentage of Infested Larvæ.

The number of infested larvæ varied to a great extent in different streams. In the part of the stream in Forest Hills where the parasites were first noticed, less than 1 per cent. of the larvæ were parasitized, half a mile further up the stream a little under 40 per cent. were infested, while in the Stony Brook Reservation where two types of "spores" were present among the parasitized larvæ, between 70 and 80 per cent. of the individuals were to be seen with the immensely distended and whitened abdomens which proclaimed them to be suffering from this fatal disease.

It will thus be seen that should this disease together with the previously described *Mermis* parasite, prove to exist as abundantly in other localities as it has during the past spring in the vicinity of Boston it must be of considerable importance in the natural control of the black flies which are such an annoyance to man and beast, especially in more tropical regions, and if the supposed secondary host of the Sporozoön does not prove to be a fish or animal of any value it should be possible to infect streams where Simuliid larvæ breed, and diminish their numbers very largely without the danger of poisoning the fish by applying oil or other substances to the water.

Summary.

Simulium larvæ, which are found in vast numbers in small rapid streams around Boston, are seen to feed by standing per-

[1] I have since July 31 noticed isolated specimens of a different species of larva in one of the streams where the parasite abounded in the spring brood, but have not found any in which there were signs of the parasite being present.

pendicularly on rocks to which they are attached by a strong anal adhesive disc, and kept in position by silken threads secreted by the salivary glands. While thus anchored they spread out a pair of cephalic fans which act as strainers and collect small particles of food from the water. The head capsule is moulted independently of the body cuticle and exposes a new capsule which is at first white with a few dark spots on the vertex, but which rapidly becomes uniformly darkened all over. The thorax bears unusually well defined and large histoblasts of the imaginal wings, halteres and legs, and also on either side a histoblast of the pupal respiratory filaments, which by turning black when the larva is mature becomes very conspicuous at this stage of growth. The larvæ are infested by two parasites, namely a *Mermis* and a Sporozoön, both of which live in the body cavity.

The *Mermis* does not affect the larval development to any extent, except by slightly increasing its size, but it inhibits the development of the histoblasts to such an extent that pupation becomes impossible.

The embryo worms are probably caught by the cephalic fans of the larvæ and pass into the alimentary tract, through the walls of which they bore and live in the body cavity of the host till the latter matures. They then rupture the abdominal cuticle and pass into the water where they live a free life under stones in the bed of the stream. The number of worms contained by a single larva is usually only one, but as many as twelve have been found. A single worm measures 3 cm., which is about three times the length of the host. In some streams 25 per cent. of the larvæ were infested with this parasite. Parasitized larvæ never pupate, but are killed by the worms when they escape.

The retardation in the development of the histoblasts is the opposite condition to that met with in prothetely which is usually caused by keeping larvæ at an abnormally high temeprature. This probably results in an increased supply of the enzymes which cause these histoblasts to develop. The *Mermis* apparently excretes some substance which lessens the supply or action of these enzymes and leads to metathetely.

The Sporozoön parasite occurs in several forms in different

localities. All these forms, however, live in the same way and appear to be related to the pébrine disease of Lepidoptera. The body, especially near the apex of the abdomen, becomes much distorted and swollen on account of its interior being closely packed with a white wooly material which on dissection is seen to consist of countless "spores" of minute size. Such parasitized larvæ are usually rather smaller than healthy individuals, but the histoblasts do not appear to be much affected. The parasite apparently enters the body cavity in the same manner as that described in the case of *Mermis*. Evidence of this is seen in a hypertrophied condition of parts of the mesenteric wall. From here it seems to pass to one or both of the sexual organs which are destroyed and become the nuclei for the great mass of spores which eventually fills the abdomen. The parasitized larvæ in this case also were never observed to pupate but died when mature. The spores are liberated by a rupture of the abdominal wall soon after the death of the host and pass into the water, after which stage they have not been seen. Up to 80 per cent. of the larvæ in some streams were found to contain large masses of this parasite but no cases of slightly parasitized larvæ were observed. There has been no second brood of *Simulium* larvæ this year, so it would seem that if the parasite is to appear next year there must be a secondary host in which the summer is passed.

Postscript.

The foregoing account of the Sporozoid parasite of *Simulium hirtipes* was very kindly reviewed for me by Professor G. N. Calkins, of Columbia University. From mounted specimens of the spores sent him he confirmed my opinion that these represent some species of Myxosporidea, and drew my attention to a paper by Louis Léger ('97), which I had overlooked because of its not being catalogued under *Simulium* in the cards of the "Concilium Bibliographicum" in the Cambridge library. In this paper a new species of *Glugea* parasitizing the larvae of the European *Simulium ornatum* is described as *G. varians*. The notes on this species are in brief as follows: The abdominal region of the infested larva is greatly distended and contains large masses of a free parasite in the form of opaque, milky white, irregular sacs.

Some larvæ contain but one mass whereas others contain two to four. The muscles are unaltered, but the fat body is much reduced. The alimentary canal always appears to be contorted. In only one case had the Microsporidea failed to sporulate and they then formed a swelling on the external intestinal surface. The sacs contain countless ovoid, refractive spores, which, when treated with iodine, show a filament 15–20 times as long as their longest diameter. Spores of two sizes are present, the smaller measuring 4–5μ, the larger 8μ. In a subsequent note written in collaboration with Hagenmueller ('08) Léger states that the spores are sometimes present in a polysporic and sometimes in an octosporic arrangement. These authors also refer to a similar parasite in the larvæ of *Tipula gigantea*.

From the foregoing notes it will be seen that the disease which occurs in *S. hirtipes* is very similar in its main features to that described by Léger, and I do not hesitate to regard the organism responsible for its occurrence as a closely related form. Professor Calkins is inclined to consider the various forms I have described as belonging to a single species. For this I would propose the name *Glugea polymorpha* sp. nov. Future investigation, however, will quite possibly show that the various forms occurring in different localities are not all representatives of the same species, since numerous dissections of diseased larvæ showed that certain types of spores were peculiar to different localities even though present in two different species of *Simulium* larvæ.

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EXPLANATION OF PLATE I.

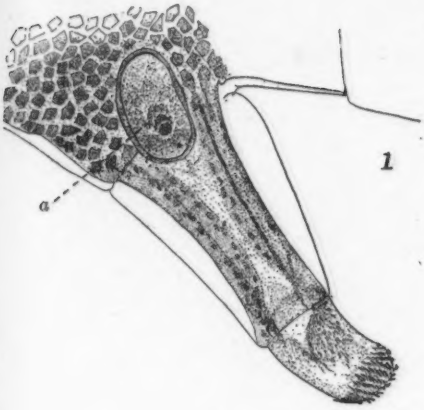
FIG. 1. Prothoracic leg of a *Simuliid* larva showing the histoblast (*a*) at an early stage of development.

FIG. 2, *a*, *b* and *c*. Types of spotting on the head of a recently moulted larva of *Simulium hirtipes*.

FIG. 3. A single rake from the cephalic fans.

FIG. 4. Profile of thorax of a half-matured larva showing the histoblasts. *a*, respiratory organ histoblast (pupal organ); *b*, wing histoblast (adult organ); *c*, halterer histoblast (adult organ); *d*, *e* and *f*, pro-, meso- and metathoracic leg histoblasts (adult organs).

FIG. 5. Ventral view of a *Simulium* larva with *Mermis* parasites in situ.



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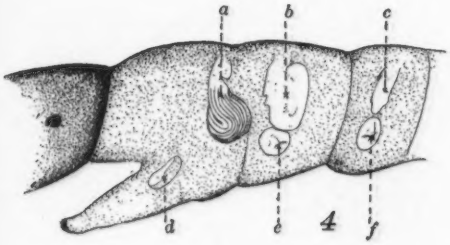

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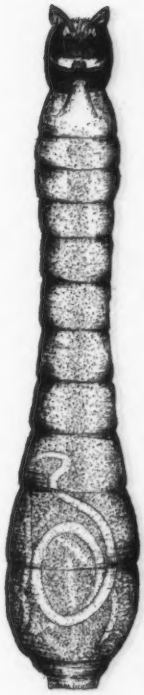

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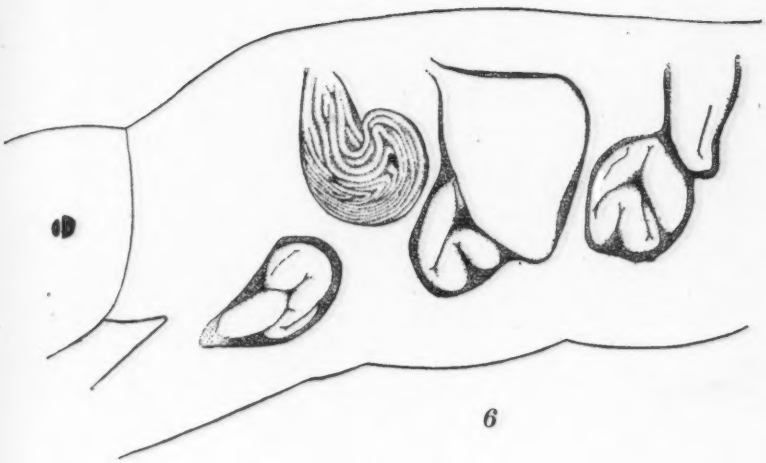
Explanation of Plate II.

Fig. 6. Histoblasts of a healthy full-grown larva measuring 10.5 mm.
Fig. 7. Histoblasts of a larva parasitized by *Mermis* measuring 10.5 mm.



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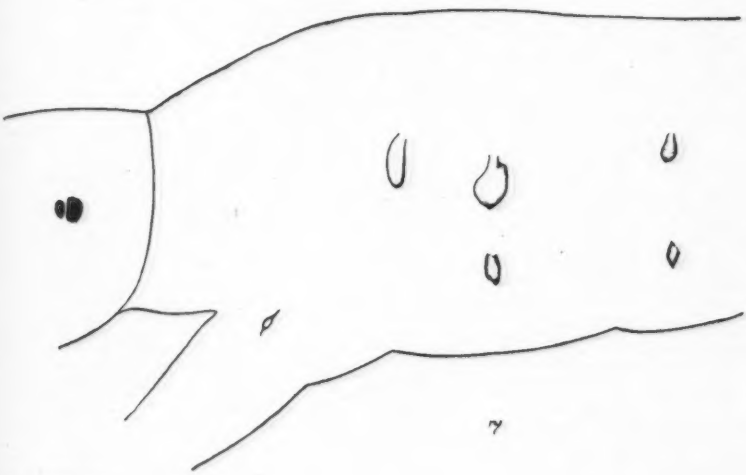

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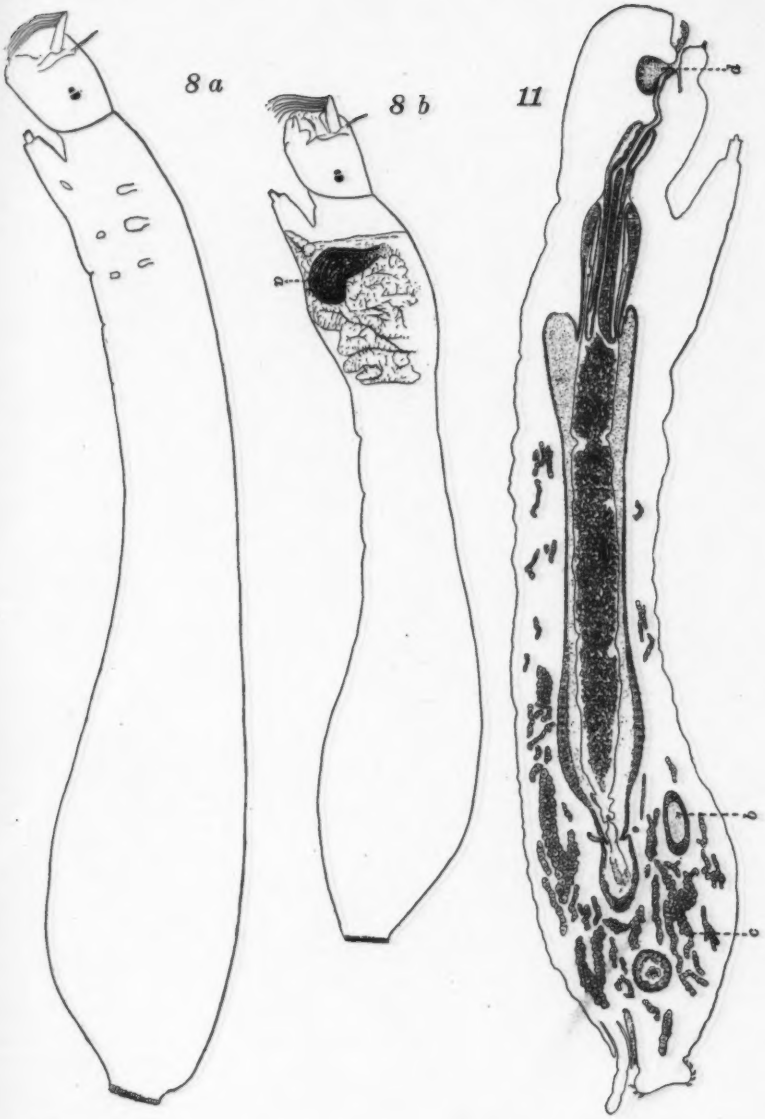
Explanation of Plate III.

Fig. 8*a*. Average size full-grown parasitized larva of *Simulium hirtipes* measuring 11 mm.

Fig. 8*b*. Average size mature larva of *Simulium hirtipes*, measuring 8.5 mm. showing darkened pupal histoblast, *a*.

Fig. 11. Median sagittal section of a half-grown healthy larva to show the alimentary tract. *b*, the normal position of the spinning glands, which extend backwards from the pharynx on either side latero-ventrally to the alimentary canal, doubling back on themselves at about the point *b*, where one has been cut in cross section; *c*, the normal quantity of fat body which increases still more as maturity approaches; *d*, one of the pharyngeal glands. (This is not seen in an exact median section, as the two glands are narrowly separated medially.)



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Explanation of Plate IV.

Fig. 9. Median sagittal section of a larva parasitized with *Mermis* sp. to show the somewhat displaced alimentary tract, and *a*, the coiled up worm; *b*, the displaced spinning gland, a short longitudinal section of which has been cut; *c*, the much reduced fat-body; *d*, one of the pharyngeal glands.

Fig. 10. Median sagittal section of a larva parasitized with a sporozoön to show *a*, the mass of spores, a few of which are shown enlarged; *b*, the spinning gland; *c*, the much reduced fat body; *d*, one of the pharyngeal glands, and *e*, the somewhat distorted wall of the alimentary tract.

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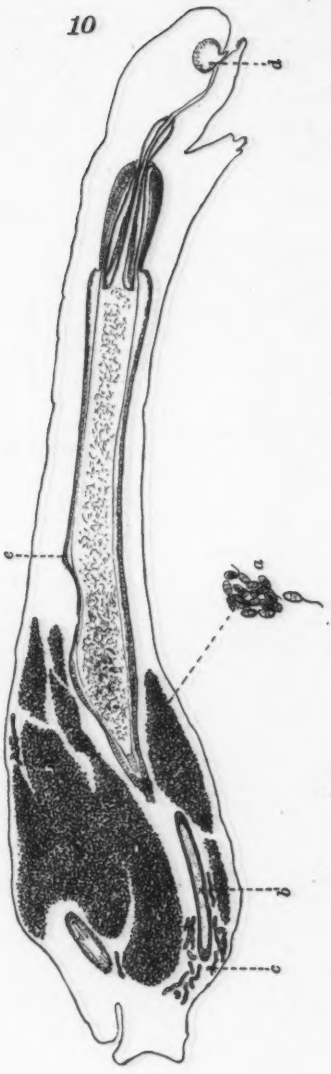

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EXPLANATION OF PLATE V.

FIG. 12. Dorsal view of a *Simulium* larva with *Sporozoid* parasites in situ. Compare with Plate I., Fig. 5, which is normal in shape.

FIG. 13. Histoblasts of a larva parasitized with the *Sporozoa* measuring 9 mm. Compare with Plate II., Fig. 6.

FIG. 14, *A*, *B* and *C*. Simple "flagellate" type of parasite, ×4,000.

FIG. 15, *A* and *B*. Annulate "flagellate" type of parasite, ×4,000.

FIG. 16. Type of parasite with "flagellum" replaced by a flattened disc, *A* showing surface view of disc, ×4,000; *B* showing side view of disc, ×4,000.

FIG. 17, *A*, *B*, *C* and *D*. Simple ovoid type of parasite, ×4,000.

FIG. 18, *A*, *B* and *C*. Other bodies found in small numbers among the "spores," ×4,000. Many are larger in proportion than those illustrated. *C* shows one apparently dividing.

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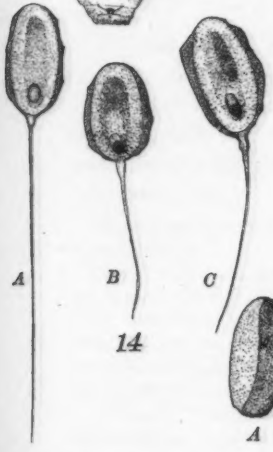
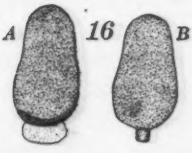


A B C

14

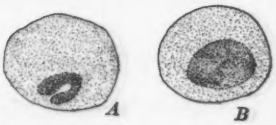
A B

15



A B

16



A B

A B C D

17

C

18

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